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EFFECTS OF ALUMINIZED FIBERGLASS ON REPRESENTATIVE CHESAPEAKE BAY MARINE ORGANISMS

SYSTEMS CONSULTANTS, INC.
1054 31st Street, N.W.
Washington, D.C. 20007

23 November 1977

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The basic static acute toxicity method was used to assess the effects of aluminized fiberglass on six representative forms of Chesapeake Bay aquatic animals. Exposure levels were derived from a simple model of the episodic introduction of the material into Chesapeake Bay, and represent multiples of up to 1000 times the expected areal exposure, with concentrations (fibers per unit volume) and durations of exposure considerably larger.

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The results indicate no significant mortality to any of the six species as a result of exposure and no accumulation of the material in the food chain, indicating that neither short-term nor long-term adverse environmental effects should occur.

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INTRODUCTION AND SUMMARY

This report describes the study of the impact of exposure to chaff upon estuarine animals. The study was conducted for the Naval Research Laboratory under Contract N00173-76-C-0307 by Systems Consultants, Inc. with the University of Delaware (College of Marine Studies) and the University of Maryland (Chesapeake Biological Laboratory) serving as the principal subcontractors.

Chaff is the collective term for aggregates of metallic or metal-coated strips or cylinders which are employed as highly efficient reflectors of radio-frequency electromagnetic radiation. The material is principally utilized in military applications as a means of degrading the performance of radars and radar-controlled weapons; chaff use has been extensive since early in World War II. Chaff has also been applied to atmospheric motion studies (Warner and Bowen, 1953; Anderson, et. al., 1956; Batten, 1958).

The Naval Research Laboratory's Chesapeake Beach, Maryland, facility is one of the principal sites of chaff experimentation. Chaff is launched from aircraft or ships at the test site, with the result that the material is introduced into Chesapeake Bay. The purpose of this study was to determine the effects of chaff upon the animals which inhabit the Bay.

During the first phase of the study, animals representative of the range of species found in Chesapeake Bay were subjected to short-term exposure to chaff. The exposure levels were determined by first calculating the typical incidence of chaff (in dipoles per square foot) at the surface of the Bay resulting from a single chaff system test event. The result was then multiplied by 100 or 1000 to determine the exposure levels for the biotic response tests. It was determined that most of the species tested (including all finfish, mussels and blue crabs) were unaffected by exposure to chaff.

A second study phase was entered principally because we were dissatisfied with the techniques used in the first study, particularly with regard to oyster larvae and polychaete worms. For example, the subcontractor used a species of polychaete worm which is not found in Chesapeake Bay. The second tests were designed to improve upon the first in several important respects including: (1) achievement of higher levels of survival among the oyster larva control groups; (2) use of a species of polychaete worm which is indigenous to Chesapeake Bay; (3) use of Chesapeake Bay water drawn from the general area of the NRL test site, and artificial sea water, both of which are of substantially better water quality than the Delaware Bay water used in the first set of experiments; and (4) experimental design more conducive to investigation of possible causes of mortality.

The second phase study indicated no adverse effects to polychaete worms resulting from exposure to chaff; although certain oyster larva tests indicated possible effects, the oyster results as a whole are consistent with the hypothesis that exposure to chaff is harmless to oyster larvae.

The remainder of this report contains a description of the methodology used for the study, a discussion of the simple exposure model utilized, a summary of the results of laboratory tests of biotic response, and conclusions, predictions and recommendation. Complete copies of the University of Delaware and University of Maryland final reports on the biotic response experiments are contained in the appendices.

This document represents the opinion of Systems Consultants, Inc. The views and conclusions set forth herein are those of the author and should not be interpreted as necessarily representing the official policies, either expressed or implied, of the subcontractors, the Naval Research Laboratory or the U.S. Government.

The contribution of R. M. Block to the section summarizing the test results is hereby acknowledged.

MATERIAL DESCRIPTION AND EXPOSURE MODEL

Physical Description

The configuration and composition of chaff are selected to produce desired electrical and mechanical properties, and to facilitate manufacture and dispensing. The physical length of the individual chaff elements is determined exclusively by electrical considerations: the electromagnetic scattering cross section is maximized for an element length approximately half the wavelength of the radar signal. At microwave frequencies (S through K_u bands) these lengths are 7.5-0.8 cm. The cross sectional shape and area of a chaff element are relatively unimportant from the electrical viewpoint, so it is desirable to make the elements as thin as possible, within mechanical constraints and manufacturing limits, to maximize the number of elements which can be packaged in a given volume. This permits achievement of larger chaff cloud cross sections or more chaff clouds of a given cross section per payload.

Aluminum is the ideal material for chaff because of its high electrical conductivity (exceeded only by gold, copper and silver) and low comparative cost. Consequently, virtually all chaff utilizes aluminum. However, the mechanical properties of aluminum are not conducive to the fabrication of very fine strands of the material. so typical microwave chaff utilizes aluminum deposited on fiberglass filaments.

Typical Navy specifications require the fabrication of aluminized fiberglass chaff having a nominal (coated) diameter of approximately 25 μm (1 mil), the aluminum coating thickness being 3.0 $\pm$1.5 μm (0.12 $\pm$.06 mil). (Naval Air Systems Command, 1971). Actual measurements of production chaff samples from the three major U.S. manufacturers (Blackburn, 1976) yielded average coated and uncoated diameters in the ranges 29-34 μm and 22-23 μm respectively,

indicating average coating thicknesses varying from manufacturer to manufacturer in the range 2.9-6.1 μm. The aggregate data (all manufacturers) are: uncoated diameter 22.9 μm (standard deviation 2.9 μm) and coated diameter 31.8 μm with standard deviation 5.8 μm.

Chemical Composition and Salt Water Chemistry

Navy specifications typically require the use of a type E glass monofilament coated with aluminum of 99.0% or greater purity. A secondary coating, generally stearic acid ($CH_3(CH_2)_{16}COOH$), is applied to the product to aid in dispersal of the chaff. According to the Department of Defense specification for fiberglass (Department of Defense, 1975) the chemical composition of type E fiberglass is:

Component	Percent by Weight
B_2O_3	5-10
CaO	16-25
Al_2O_3	12-16
SiO_2	52-56
MgO	0-5
Na_2O and K_2O	0-2
TiO_2	0-0.8
Fe_2O_3	0.05-0.04
F_2	0-1.0

Analysis of chaff samples from all three major manufacturers verified that the fiberglass used contained levels of Fe, Al and Mg which were all within the limits specified above (Roginski, 1976). The aluminum coating material used by the three major manufacturers was also analyzed independently by three groups (Venezky, 1977; Roginski, 1976; and Blackburn, 1976). All groups' results confirmed compliance to the 99.0% purity requirement for the aluminum

material; the quantitative results of Venezky and Roginski indicated a minimum aluminum purity of over 99.75%. The remaining constituents mainly included Fe (0.12 to 0.20%), with traces of other metals (typical results: Zn - 100 ppm; Cu - 26 ppm; Ni - 14 ppm; Mn - 10 ppm; Cr - 7 ppm; Cd - 1 ppm). On the average, the chaff samples consisted of 45.6% metals, 44.3% fiberglass and 10.1% stearic acid by weight.

Venezky placed multiple chaff samples in water drawn from the Chesapeake Bay test site area in December 1976. After 13 days in solution, the water was analyzed for Al, Cd, Cu, Fe and Zn. No appreciable (1 ppm) increase in concentration of any of these metals in the water was noted; in fact, most increased by only a few tens of ppb.

Chaff Aerodynamics

The mobility of chaff in the atmosphere is well characterized, with empirical results in good agreement with theoretical predictions (Jiusto and Eadie, 1963). Thus the general characteristics of chaff motion within water are expected to be easily predictable. However, since the individual chaff dipoles are small and of modest density, they do not readily overcome surface tension, and consequently might float for some time after reaching the water surface from the air. Such an effect would naturally tend to increase the area over which chaff was dispersed. Owing to the general lack of detailed information concerning the phenomenon, and also uncertainties regarding currents in Chesapeake Bay, the study utilized the simplest of all possible models for determining chaff exposure levels. The purpose of this subsection is to introduce the models by indicating general characteristics of chaff motion in the atmosphere at the Chesapeake Beach test site, and to extend that model by characterizing the expected dispersion in chaff concentration taking place in Chesapeake Bay.

The Jiusto and Eadie formula for chaff terminal velocity in still air is:

$$V_T=\left(\frac{\pi\rho_c g}{21}\right)^{0.73} d^{1.19}/\mu^{0.46}\rho_a^{0.27}$$

where ρ_c is the chaff density (typically 2.59 g/cm^3), g the acceleration of gravity, d the chaff diameter, μ the dynamic visiosity of air and ρ_a its density. From the equation it is seen that variation in the dipole diameter will affect the terminal velocity and thus result in vertical dispersion of a chaff cloud in still air. In the presence of winds, a lateral dispersion of the chaff will result even in the absence of local atmospheric turbulence because of this. Based on the results of Blackburn, the median fall rate of typical production chaff at low altitudes and under standard conditions is 26.3 cm/s with 1σ limits of 20.7 cm/s and 32.2 cm/s, yielding a 1σ vertical dispersion rate of 11.5 cm/s under still air conditions. In the presence of a horizontal wind velocity component V, the 1σ horizontal dispersion would then be 1.725 V centimeters per meter of fall, where V is in cm/s. We have examined surface wind speed data available for Annapolis and Lexington Park, Maryland, locations which geographically bracket the Chesapeake Beach chaff test site. Seasonal average surface wind speeds at these locations were 8.1 and 8.2 knots, respectively. Thus it is estimated that the typical 1σ horizontal dispersion rate for chaff near the surface at Chesapeake Beach is 720 cm per meter fall. This corresponds approximately to the empirically derived model provided by NRL for estimating chaff exposures (see the following subsection). It is noted that wind velocities tend to increase with altitude, and local atmospheric motion irregularities (e.g. updrafts) frequently exist over Chesapeake Bay. These factors tend to increase the horizontal dispersion of chaff in the atmosphere.

As indicated previously, chaff has a tendency to float on the surface of the water to an extent which is influenced by numerous factors, the most notable of which is the state of the surface itself. During the tests

discussed in the appendices, for example, the experimenters consistently experienced difficulty inducing the material to sink despite stirring, etc. This property results in dispersion of the material in the environment over and above that which takes place due to chaff aerodynamics: the chaff reaches the surface of Chesapeake Bay over a period of time so that currents in the Bay will cause a spreading of the chaff pattern on the surface.

Once the surface of the Bay is broken by a chaff element, it will sink. The terminal velocity can be calculated using

$$V_T=\left(\frac{\pi g}{21}(1-\rho_w/\rho_c)\right)^{0.73}(\rho_w/\mu)^{0.46}d^{1.19}$$

where ρ_w is the water density and μ is its dynamic visiosity. This equation includes the chaff bouyant force, which can be neglected in the corresponding equation for air. For typical chaff, the mean terminal velocity at a depth of about 1 m is 2.4 mm/s; the 1σ limits are 1.9 mm/s and 2.9 mm/s. The average low tide depth of Chesapeake Bay is approximately 8.4 m, so that the average time required for chaff to sink to the bottom is on the order of 60 minutes. During the sinking process, the chaff is further dispersed by the complex currents of the Bay.

<u>NRL Exposure Model</u>

Navy tests at the Chesapeake Beach test site involve a variety of chaff dispensing systems; for the purposes of this work, however, they may be grouped into two categories: aircraft-launched and surface-launched.

In a typical test of an airborne system, multi-frequency chaff ranging from 16 mm to 51 mm in length is dispensed from ALE-41 pod dispensers in a single aircraft. The flight corridor is approximately 1800 m wide, 18.5 km long and 1500 m thick at drop altitude. Under estimated prevailing wind conditions of 35 knots at the drop altitude the chaff remains aloft one to one

and one half hours and is estimated to impact surface in an extremely thin footprint pattern within a 140 km radius of the dispensing location.

Evaluations employing airborne systems are predicted to continue at an average rate of one flight test each month, wherein an average quantity of 36 kg of chaff will be dispensed per flight. The following constitutes the calculation of the chaff concentration in the air ten minutes after dispensing:

(a) Dispenser utilized: ALE-41

(b) Chaff weight: 40 lbs/roll or 0.40 lbs/ft. (100 foot rolls)

(c) Chaff dipole count/ft.: 35×10^6/ft.

(d) Dispensing rate: 8 inch/sec.

(e) Chaff dipole count/in: $.029 \times 10^8$/inch

(f) Aircraft Speed: 350 knots (591 ft./sec.)

(g) Dispensed Chaff at Altitude immediately behind aircraft (1 sec.): Past test experience has shown that the chaff pattern spreads to approximately 100 feet in width in approximately 1 second.

$$\frac{\frac{8 \times .029 \times 10^8}{591}}{10^2} = .0395 \times 10^4 \text{ dipoles/ft.}$$

(h) Dipole density approximately 10 minutes after dispensing: Pattern continues to spread (lateral creep) at rate of 1 foot per each 1-1/2 feet of drop. Maximum rate of drop is calculated at 2 feet/second. The corridor will expand to an area of approximately 900 feet wide and 100 feet in height approximately 10 minutes after dispensing. The dipole density would then be .0044 dipoles/cu. ft.

During the evaluation of surface ship chaff launching systems, multi-frequency chaff similar to that described above is introduced into the atmosphere at low altitude by a mortar launching system mounted on a boat.

The dispersing area is approximately 900 yards offshore within a 25° included angle bearing from the radar tracking site (approximately a 400 yard leg perpendicular to the tracking site). The dispersing burst is at approximately 400 feet altitude. Under no updraft wind conditions the chaff dipoles would remain aloft for 200 seconds and are estimated to impact surface with a footprint density of 53 dipoles per square foot of area as calculated below.

Evaluations employing this mode are predicted to continue at an average rate of two test firings each month (approximately 20 rounds/firing) wherein an average quantity of 110 kg of chaff will be dispensed each month.

The following calculations were utilized to arrive at the chaff exposure levels at the surface of the Bay resulting from a surface ship chaff launching system test:

(a) Dispenser utilized: MK 133 mortar round

(b) Dipole count: $.1065\times10^{8}$ dipoles/average round

(c) Burst altitude: 400 feet

(d) Dipole bloom diameter: 30 feet dia.

(e) Dispensed Chaff at Altitude:

$$\frac{.1065\times10^{9}}{\pi(1.5\times10^{2})^{2}\times(3.0\times10^{2})} = 5.02 \text{ dipoles/cu. ft.}$$

(f) Dipole density at impact: Past test experience has shown that the chaff pattern spreads (radial creep) at rate of approximately 5 rad. ft./sec. through initial 60 seconds of dispersement life. Radial creep continues at rate of approximately 2-1/2 rad. ft./sec. for balance of drop. Maximum rate of drop is calculated at 2 ft./sec. The Bloom diameter will expand to approximately 1600 feet mean dia. at impact in a still air wind condition.

$$\frac{.1065\times10^{9}}{\pi(8.0\times10^{2})^{2}} = 53 \text{ dipoles/sq. ft. at impact}$$

On the basis of the foregoing it can be seen that testing of the tw- types of chaff systems will result in vastly different levels of exposure and scale even at a dispensing altitude of only 1200 f the airborne system yields a chaff density which is three orders of magnitude smaller than that due to a surface system, with a corresponding increase in exposure area. Consequently, only the surface system exposure level was used for the biotic response tests. The models yield fallout levels at the surface, which would be adequate if the tests were conducted over land. In reality, the material falling on Chesapeake Bay will be further dispersed in the water column, so that specification of exposure in terms of dipoles per unit area is inappropriate unless the dispersion in the Bay is neglected and only bottom living animals are considered. For fish and oyster larvae, it is necessary to calculate exposures in dipoles per unit volume; however, limitations to the scope of the contract precluded accomplishing this. In any case, the limitations of the model and the static nature of the biotic response tests clearly result in laboratory exposure levels which are far in excess of those which are encountered in the actual environment, even for those lab test events conducted at the 1X (53 dipoles/f^2) exposure level.

METHODOLOGY

A variety of estuarine organisms was selected for testing th possible impact of chaff to the environment. These species included organisms that were either ecologically, sport or commercially, significant. Each represents the dominant biomass for finfish and shellfish found in the area subjected to chaff deployment. These species include the benthic polychaete worm, Nereis succinea, the various life stages of the American oyster, Crassostrea virginica, the blue mussel, Mytilus edulis, the blue crab, Callinectes sapidus (of which only the juvenile and adult stages are found in Maryland's waters) as well as the filter feeding mehaden, Brevoortia tyrannus and the killifish, Fundulus heteroclitus. Since turnover rates of phytoplankton and zooplankton are so rapid in the Chesapeake Bay system, representative species of these latter two groups are not tested.

All experiments with chaff exposure to the selected estuarine species were conducted in static systems producing a worst-case situation. High exposure levels of chaff were also used to determine if more sophisticated toxicity screening tests would be needed in subsequent investigations.

It is well known that motile animals such as finfish and crabs will behaviorally avoid a toxic influence. However, since little is known about behavior avoidance reactions of fish or crabs to chaff, a worst-case situation was produced by actually force-feeding these species both fractionated and long form chaff fibers. Both lethal and sublethal indicators of stress due to chaff were investigated. These included mortality, growth rate, behavioral modification and histological examination that would be indications of physiological deterioration that would eventually effect the growth and/or reproductive ability of an organism.

SUMMARY OF LABORATORY TEST RESULTS

Reported below are the bioassay results on the effect of chaff to selected estuarine species found in the Chesapeake Bay. Tests were performed by the University of Delaware (six species) and the University of Maryland (two species). The results of these tests are presented and discussed on a species-by-species basis.

American Oyster (*Crassostrea virginica*)

Since the oyster is one of the most commercially important benthic shellfish found in the Chesapeake Bay, this was the most extensively tested species. In general, oysters are benthic, non-motile filter feeders. They inhabit saline waters from 5 to 30 $^{o}/oo$ salinity. In Maryland waters, oysters spawn at water temperatures of about 22-28^{o}C which can occur as early as April and as late as July. In order to determine the most sensitive life stage of oysters to chaff, three stages of development were investigated: 0-48 embryonic oysters, 10 day old larvae and juvenile oysters (1.7 to 2.4 cm). Studies on all three life stages were conducted by the University of Delaware. Due to improper techniques employed by the University of Delaware (as indicated by low control group survival rates) for the 0-48 embryonic oyster studies, investigations on these animals were performed by the University of Maryland at a later date.

1. Embryonic Oyster Studies (0-48 hours)

A. University of Delaware Results

The embryonic larvae showed 100% mortality at both the 100X and 1000X exposure levels to chaff in either the long or fractionalized form. The control groups experienced 70-80% mortality for the 48 hour experiment. Accurate counts of live versus dead larvae could not be made, and statistical tests could not be performed on t. 'ata. This study was conducted at 28 $^{o}/oo$

salinity at 26°C, a normal temperature for the occurrence of oyster spawning. We consider these results invalid for two reasons: extreme mortality of controls suggests that impurities may have existed in the experimental water (this subject is discussed below in detail), and 30 years of records of salinity and temperature in the Solomons' area have shown the maximum salinity to be 16 ‰ while the minimum recorded has been as low as 7.6 ‰ during ambient temperatures of 26°C (Ritchie and Genys, 1975). For these reasons, the 48 hour oyster larvae experiments were repeated by the University of Maryland at Solomons, Maryland.

B. University of Maryland Results

Detailed description of the methodology can be found in Appendix B. One distinctive advantage of the method used in these studies was that the entire larvae population of each experimental tank was counted rather than simply using a 1 ml aliquot of larvae, as was used in the University of Delaware experiments. The salinity range during these experiments was from 13.2-15.3 ‰ All experiments were run in triplicate.

The first set of experiments was a comparison of Chesapeake Bay water versus synthetic seawater using larvae exposed to either aluminum coated or non-coated chaff material at a 10X and 100X exposure level. A 5/8" length of chaff was used for all tests. The results of these experiments clearly indicate that the Chesapeake Bay water provides a better media for 0-48 hour oyster larvae than synthetic seawater. This may be explained by the fact that the ratio of ions in estuarine waters is different from that found in diluted seawater at the same salinity. Of greatest importance is that the Bay is extremely rich in organic carbon and other nutrients not found in synthetic seawater or marine waters. Therefore, the best toxicity data relating to the actual environment can be obtained using as natural water as possible where the toxicant in question may be a possible threat to the environment.

No statistical interpretation could be made from the first set of experiments since one test chamber in each test situation was used to monitor water quality. Apparently contamination resulted in the beaker causing increased mortality to larvae that would not have occurred under normal conditions. However, based on superficial examination of the results of these experiments, a question was posed as to whether the toxicity of chaff is from larvae contact with the material itself or simply from the presence of the material in a static water column.

The University of Maryland experiments were set up similarly to Delaware's except 1X and 10X exposure levels of chaff were used in either an aluminized or non-aluminized form. Also, one set of tests utilized larvae in an uncaged (direct contact) or caged situation (indirect contact with chaff). All experiments were run in triplicate.

Although two methods were used to determine percentage mortaility of oyster larvae, we have elected to interpret the results based on the method by Calabrese, et al (1973). This method is recommended by Standard Method (1976). No significant effect ($P \leq 0.05$) of chaff to oyster larvae was observed in any of the uncaged experiments except for the 10X non-aluminized sample. Since chaff is not deployed in the non-aluminized form, we can readily reject this result as having any environmental consequences.

In the caged studies, a significant ($P \leq 0.05$) effect of chaff to oyster larvae was observed at the 1X aluminized exposure level, but not at the 10X exposure level. These results are inconclusive since it would be impossible for chaff to exert a greater toxicity at a lower exposure level than at the higher exposure level. In summary, both aluminized and non-aluminized chaff, either by direct or indirect exposure to oyster larvae, has no toxic influence on 0-48 hour oyster embryos in a static system using Chesapeake Bay water.

2. Pediveliger Stage Larvae (10 day old larvae)

Ten day old oyster larvae were exposed to chaff for 48 hours in a manner similar to the embryonic larvae studies. During this stage of development larvae swim freely in the water. The results of these experiments are summarized in Table 1.

These data indicate that the long form of chaff has no significant effect ($P \leq 0.05$) on the survival of 10-12 day old larvae at exposure levels up to 1000X. Similarly the fractionated chaff did not significantly effect larval densities at 1000X exposure level. It is interesting to note that larval densities were significantly enhanced after 48 hour exposure to 100X fractionated chaff. Fractionated chaff had no effect on the larval size; however, the long form did significantly decrease the size from the control at the 1000X exposure level. Since size is an inverse function of density of population, the addition of the 1000X chaff could simply have inhibited size increase of larvae as a result of the reduced space in the closed experimental test chambers. The overall physical density within the test chamber was increased due to the suspended nature of the chaff in water. This would be a situation that oyster larvae would not encounter in the environment. It is concluded that the chaff would have no effect on either the survival or development of 10-12 day old larvae.

3. Juvenile Oysters

Both feeding and growth experiments were conducted on oysters (1.7 to 2.4 cm in length) exposed to long and fractionated chaff at 100X and 1000X exposure levels. Details of these studies are described in Appendix A (pp. 15-17). Of the 16 experiments conducted on algal uptake (feeding) into oysters, only three showed significant ($P \leq 0.05$) differences (1000X) from the controls. However, when data from all experiments were pooled, no significant effects of chaff on the feeding rates of oysters were observed after three weeks of exposure. Chaff had no significant effect on oyster growth after four weeks of exposure.

TABLE 1

Summary of Results of Exposure Tests for 10 Day Old American Oysters

Test	Sample Mean	F (vs. Control)
MEAN LARVAL DENSITY (Sample Count)		
Long Form Control	106.5	-
Long Form 100X	117.8	N.S.
Long Form 1000X	97.8	N.S.
Fractionated Control	92	-
Fractionated 100X	157.5	8.819*
Fractionated 1000X	92.3	N.S.
LARVAL SIZE (Micrometers)		
Long Form Control	197.7	-
Long Form 100X	185.1	N.S.
Long Form 1000X	175.6	34.4*
Fractionated Control	187.4	-
Fractionated 100X	187.3	N.S.
Fractionated 1000X	185.3	N.S.

*Significant F value at 95% Confidence Level

N.S. - Not Significant

Although the University of Delaware reported a significant decrease of growth of oysters exposed to chaff at the 100X level, no significance was observed at the 1000X exposure level. This inconsistency may be explained by the fact that statistical analysis was made only within a group population rather than statistical comparison of test animals versus controls. Overall, one can conclude a wide variation of growth within the sample population invalidating any inferences on the effects of chaff on oyster growth. Histological studies indicated food assimulation into chaff exposed oysters as well as controls. No cellular damage was found in any of the experimental groups.

4. Juvenile Oysters - Trace Metal Studies

In general, at the pH of the Chesapeake Bay,* metal ions such as Al, Cu, Zn and Cd are converted to the insoluble hydroxides or undergo ion-exchange with colloidal clay particles and ultimately sink to the bottom (Helz, pers. communication). Of the benthic invertebrates, mollusks (particularly the American Eastern oyster) has the greatest propensity for trace metal uptake, particularly of Zn, Cu and Cd (Pringle and Shuster, 1967). Oysters can entrap particles as small as 1.0 μ diameter and the average size of the particle trapped is about 3 μ (Haven and Morales - Alamo, 1968). These animals can absorb metal ions from the entrapped colloidal material and eliminate the ingesta into feces and pseudofeces. Consequently, the greatest source of heavy metal uptake into oysters is from suspended material found near the bottom of the Chesapeake Bay. A study completed by the U.S. Environmental Protection Agency has shown the sediments from the Baltimore Harbor area contains 50 times more copper, 7 times more zinc and 6 times more cadium than sediments found in the Chesapeake Beach area (Villa and Johnson, 1974). Helz (1976) completed an inventory of trace elements in the Northern Chesapeake Bay and concluded that the principal source

*The Ph of Chesapeake Bay is typically 8.

of Cu is direct industrial discharge; for Cd from municipal wastewater while Zn is from river discharge, shore erosion and saltwater advection from the oceans.

An extensive field study was conducted during 1970 from July to October on the uptake of Cu and Zn into oysters at three locations on the Chesapeake Bay (Figure 1) as well as the concentrations of these metals in both the water column and in sediment (Cronin, et al, 1974). Table 2 provides a comparison of Cu and Zn values actually found in the Chesapeake Bay and those concentrations used in the oyster larval studies. The concentration of copper used in the University of Delaware oyster larval studies was about three times higher than the Cu concentrations found in the Chesapeake Bay where chaff may be deployed. Zinc concentrations were about 2.5 times greater in the experimental water than actually found in the Chesapeake Bay. A comparison of Tables 3 and 4 provides a comparison of Cu, Zn and Cd levels in oysters from the Chesapeake Bay, Delaware Bay and control oysters used in the University of Delaware studies. Data from Cronin, et al (1974) indicate that oysters rapidly accumulate Cu and Zn over a 15 day period. The levels of Cu and Zn in the University of Delaware mariculture water may explain the uptake of Cu and Zn into juvenile oysters during the 25 day testing period. Little is known about aluminum uptake into oysters or dispersion in the Chesapeake Bay. It is interesting to note that control oysters used in the University of Delaware study had aluminum concentrations from 90 to 188 ppm, twice as high as those found in the Delaware Bay oysters. Also, control juvenile oysters used in the chaff study had copper levels 11 times higher than those found naturally in the Delaware Bay oysters while Zn levels were about 2X higher than those found in their natural environment. The only conceivable explanation of these differences could be heavy metal contamination of University of Delaware testing water and the ability

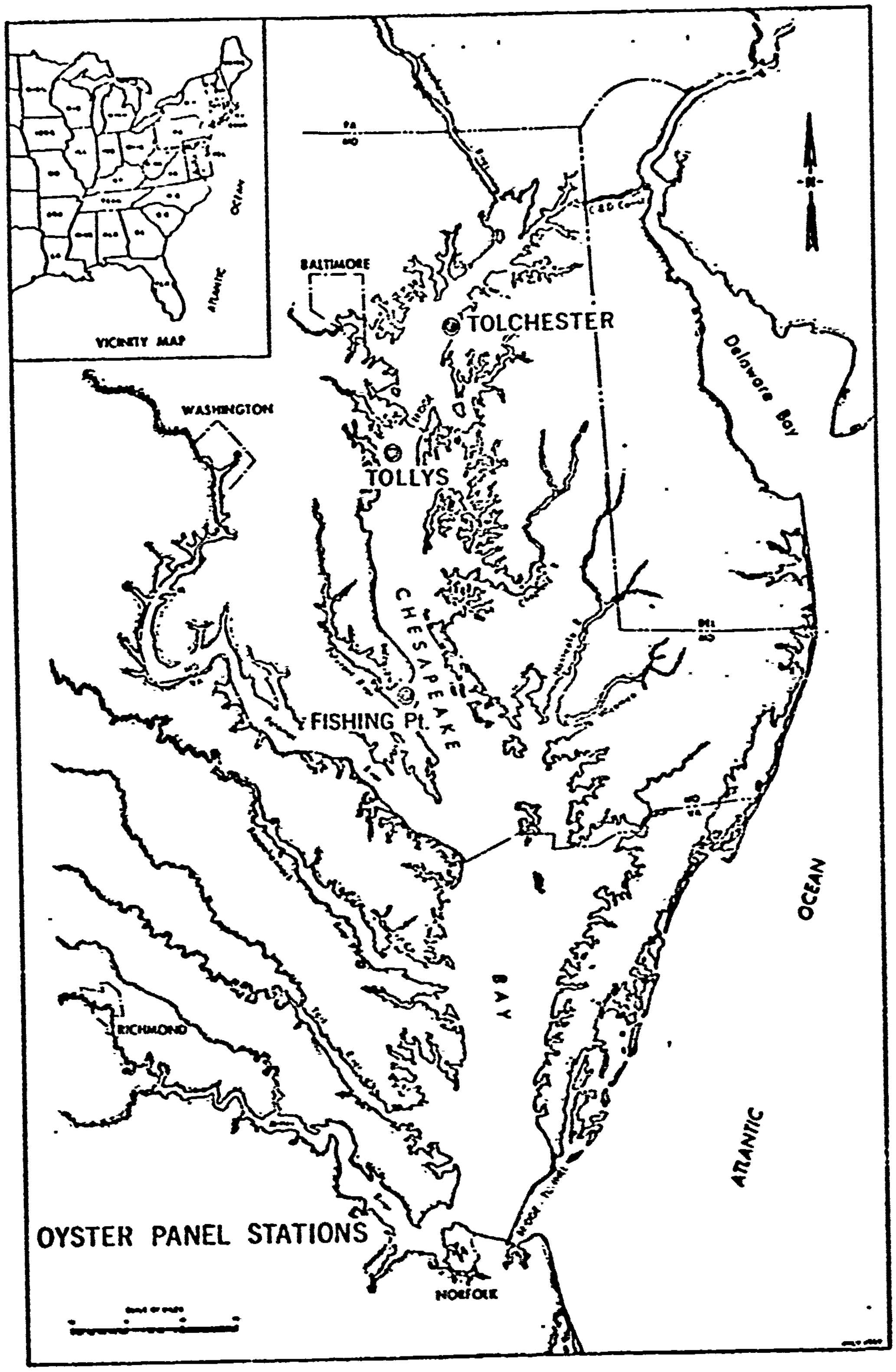

Figure 1. Sites of Stations for Exposure of Oysters

TABLE 2

Metal Analyses of Water Used in Oyster Larval Bioassay Compared with Water from the Chesapeake Bay[1]

(Numbers are in mg/L)

	Cu	Zn	Cd
Fishing Point	2.0	6.4	
Tollys	3.1	12.5	
Tolchester	4.1	13.9	
Seawater, Univ. of Del. Mariculture Project	9.0	31	0.28
Control Tank	2.4	81	1.5

1. From Cronin, et al (1974). These values are from water samples taken from 12 to 72" above the bottom during 1970.

TABLE 3

Average Concentration of Heavy Metals in Oysters 12-72 Inches from the Bottom[1]

Date	Station	Oysters µg/g-dried Cu	Zn	Cd
ca. 1 July	Tolchester	65	825	
	Tollys	65	825	
	Fishing Point	65	825	
ca. 15 July	Tolchester	297	2567	29
	Tollys	157	2167	13
	Fishing Point	63	947	7
ca. 12 August	Tolchester	390	5033	27
	Tollys	177	3000	8
	Fishing Point	73	1700	3
ca. 15 September	Tolchester	310	3533	20
	Tollys	133	2233	7
	Fishing Point	57	1400	2
ca. 21 October	Tolchester	523	5600	23
	Tollys	200	3567	7
	Fishing Point	67	2000	3

1. From Cronin, et al (1974). Oysters were all taken from the Fishing Point area on July 1, 1970.

TABLE 4

Metal Analyses of Juvenile Oysters
from University of Delaware Control Tanks

(Values are Expressed as μg/g - Dried after 25 Day Exposure)

	Cu	Zn	Cd
Control I	382	989	0.70
Control II	381	972	0.68
Adult Oysters from Delaware Bay	35	486	0.77

of oysters to bioaccumulate heavy metals from ambient water. This would invalidate any consideration that the University of Delaware results were due to leaching of heavy metals from chaff itself into the testing water.

Calabrese, et al (1973) has shown that the 48 hour LC0 for aluminum to oyster larvae was 7.5 mg/l. Our calculations on the amount of almuinum that could be present in the testing tanks would be considerably lower than 7.5 mg/l, a concentration of aluminum which produced no observable mortality in Calabrese's study. In conclusion, neither aluminum nor the other heavy metals investigated during this study could have come from chaff, but rather was a direct result of the water used during the toxicity studies.

Blue Mussels (*Mytilus edulis*)

Adult blue mussels were exposed to 100X and 1000X concentrations of chaff for a period of 21 days. No significant mortality occurred nor was any change in weight of the mussels observed after the exposure period. Feeding studies were not performed on this species.

Polycheate Worms (*Nereis succinea* and *Hydroides dianthus*)

Investigations performed on *H. dianthus* by the University of Delaware are considered to be non-relevant to areas of the Chesapeake Bay which might be impacted by the deployment of chaff. This polycheate species has never been observed in the normal range of salinties found from Chesapeake Beach to Lexington Park, Maryland.

The polycheate worm, *Nereis succinea* represents the greatest biomass of any benthic invertebrate found in the area subject to exposure from chaff in the Chesapeake Bay. Chaff exposure tests were run at Solomons, Maryland by the University of Maryland. Test water used in the studies was similar to water in the Chesapeake Beach area.

Two sets of tests were performed; four day and eight day exposure to 10X chaff. The design matrix was to expose N. succinea to aluminized and non-aluminized chaff in both a caged (not in contact with chaff) and uncaged (contact with chaff) situation. All experiments were run in triplicate. No mortality of the worms could be attributed to chaff in any of the tests. Some of the worms exposed to chaff in the uncaged experiments would actively accumulate the chaff and plug the hole of their tube with the material. This behavior response was quite random and therefore no inference can be made. However, if chaff, either in the aluminized or non-aluminized form, was indeed toxic to the worms, an avoidence response would have been observed.

Blue Crab (Callinectes sapidus)

Feeding experiments were conducted on blue crabs over a 21 day period. Each crab was fed an amount of chaff (8.43 mg/day or 84.3 mg/day) mixed with a food gel preparation. The total amount of chaff fed to the crabs would equal a 100X or 1000X level of chaff at the end of the 21 day period. No mortality due to chaff ingestion was observed during the course of the experiment. There was a significant avoidance response of the crabs to food containing chaff. Since none of the finfish or shellfish experiments indicated accumulation of chaff into animal tissues, crabs would not be subjected to chaff ingestion in the environment under worst-case situations. Histological studies of the digestive tract showed no apparent pathology that would result in physiological abnormalities due to chaff ingestion.

Menhaden (Brevoortia tyrannus)

The filter-feeding juvenile menhaden were exposed to chaff of various lengths which was added directly to the water. Groups of 27 fish were exposed to 100X and 1000X chaff for 21 days. No significant mortality or weight change

occurred in these fish exposed to chaff when compared to control fish. Histological examination of the stomach and intestines of both control and experimental fish showed no presence of chaff nor cellular damage. Food was found in the digestive organs of both groups of fish. This would indicate that menhaden did not ingest chaff nor were their feeding habits greatly altered by the presence of chaff in the test water.

Killifish (Fundulus heteroclitus)

The effect of chaff on the killifish was determined by feeding experiments. Chaff was added to fish chow in a manner similar to the blue crab feeding studies. The daily amount of chaff added to the food would produce a 100X or 1000X ingestion level at the end of the experimental period assuming all fish ingested 100% of their daily ration. The fish were separated into three groups according to size (small, medium and large). Small, medium and large fish were exposed to chaff for three, four and five weeks respectively.

No significant differences of mortality, weight and length changes were observed for test fish compared to controls. Histological studies on digestive organs failed to produce any pathological aberations due to chaff ingestion.

CONCLUSIONS AND RECOMMENDATIONS

Achievement of the purpose of this study - to determine whether the testing of chaff systems at Chesapeake Beach will have any adverse effect upon the environment - makes it essential to distinguish between the environments of the laboratory and the Bay. The two most important differences between the test environment and the actual Bay system involve the absence of natural effects in the laboratory which would both dilute the concentration of chaff and the exposure duration to it, and the use in the laboratory of unrealisticly high concentrations of chaff. This second factor was included deliberately to accelerate any adverse effects, but because of the model used the resulting exposure levels were higher than indicated. Let us discuss a case in point: the 0-48 hour oyster larvae experiments. In the University of Maryland tests, 1X and 10X the 53 dipoles/ft^2 exposure were used in beakers having surface areas of 0.6 f^2 and volumes of 1.0 l. Thus the concentrations of dipoles were 32 dipoles/liter and 318 dipoles/liter. The average depth of Chesapeake Bay is 8.4 m, so that the nominal concentration of chaff in the water column due to 1X and 10X fallout levels would be 53 dipoles/0.78m^3 = 0.068 dipoles/liter and 0.68 dipoles/liter, respectively, assuming no flushing of the chaff due to currents. Thus the so-called 1X exposure nominally represents 471 times the average concentration of chaff in the water column under worst-case conditions. For benthic organisms such as juvenile and adult oysters, mussels, crabs and polychaete worms, specification of exposure levels in dipoles/ft^2 is less unpalitable than for pelagic organisms such as oyster larvae and finfish.

Taking these factors into account, SCI concludes that the laboratory tests support the contention that the testing of chaff, as presently accomplished, will have no environmental impact upon the Chesapeake Bay ecology. Although certain of the test results are subject to various interpretations, the application of scaling factors when relating those results to the environment mitigate against predicting adverse effects. We see no reason for continued toxicity testing of chaff to aquatic organisms, except possibly to satisfy scientific curiosity. We recommend that the chaff test program over Chesapeake Bay be allowed to continue.

LITERATURE CITED

American Public Health Association, American Water Works Association and Water Pollution Control Federation: Standard Methods for the Examination of Water and Waste Water, 14th Ed. 1193 pp., Washington, D.C., 1976.

Anderson, A.D. and W.E. Hoehne, 1956. Experiments using window to measure high altitude winds. Am. Met. Soc. Bul. 37: 454-457.

Battan, L.J., 1958. Use of chaff for wind measurements. Am. Met. Soc. Bul. 39: 258-260.

Blackburn, E.C., T.L. Bowen, E.L. Fyrnys and D.A. Werner, 1976. Aluminized glass chaff, Lundy, Tracor, and M.B. Associates; evaluation of relative properties. Naval Avionics Facility Materials Test Report 79-76.

Block, R.M. G.R. Helz and W.P. Davis, 1977. The fate and effects of chlorine in coastal waters. Ches. Sci. 18: 97-101.

Calabrese, A., R.S. Collier, D.A. Nelson, and J.R. MacInnes, 1973. The toxicity of heavy metals to embryos of the American Oyster *Crassostrea virginica*. Mar. Biol. 18: 162-166.

Cronin, L.E., D.W. Pritchard, J.R. Schubel and J.A. Sherk, 1974. Metals in Baltimore Harbor and Upper Chesapeake Bay and their accumulation by oysters. Phase I. Unpublished progress report, 87 pp. Chesapeake Bay Institute, Johns Hopkins University, and Chesapeake Biological Laboratory, University of Maryland.

Department of Defense, 1975. Military specification roving glass, fibrous (for filament winding applications) MIL-R-60346B.

Haven, D.S. and R. Morales-Alamo, 1968. Va. J. Sci 19: 178.

Helz, G.R. 1976. Trace element inventory for the Northern Chesapeake Bay with emphasis on the influence of man. Geochim Cosmochim Acta. 46: 573-580.

_____, 1977. Private communication.

Jiusto, J.E. and W.J. Eadie, 1963. Terminal fall velocity of radar chaff. J. Geophys. Res. 68: 2858-2861.

Mountford, N., 1977. Private communication.

Naval Air Systems Command, 1971. Military Specification chaff, countermeasures RR-129/ALE 29A MIL-C-81467B(AS).

Pringle, B.H. and C.N. Shuster, 1967. A guide to trace metal levels in shellfish. Shellfish Sanitation Technical Report. North Marine Health Sciences Laboratory. Nattagansett, Rhode Island, 18 pp.

Ritchie, D.E. and J.B. Genys, 1975. Daily temperature and salinity of surface water of Patuxent River at Solomons, Maryland, based on 30 years of records (1938-1967). Ches. Sci. 16: 127-133.

Roginski, T.C., 1976. Private communication.

Venezky, D.L., 1977. Characteristics of chaff in contact with pure water and Chesapeake Bay water. Naval Research Laboratory memorandum 6130-39: DLV: cmc.

Villa, O. and P.G. Johnson, 1974. Distribution of metals in Baltimore Harbor sidements. Technical Report 59. U.S. Environmental Protection Agency, Annapolis Field Office, Region III.

Warner, J. and E.G. Bowen, 1953. A new method of studying the fine structure of air movements in the free atmosphere. Tellus 5: 36-41.

APPENDIX I

The Biotic Respone of Typical Estuarine Organisms to Aluminized Fiberglass Chaff

Report to:
Systems Consultants, Inc.

by

Richard T. Keck
Wayne A. Leathem
Jeffrey C. Tinsman
Betsy Brown
Pieter Kat
Don Maurer
Lowell Sick

University of Delaware
College of Marine Studies
Lewes, Delaware 19958

21 March 1977

Introduction

The United States Navy has employed the use of aluminum-coated fiberglass (chaff) dipoles as an airborne self-protection system. The chaff has been used effectively as protection against weapons and radar systems. The material is typically deployed on the surface or at low altitudes over water. It has been estimated that fallout from chaff deployment results in an average concentration of 53 dipoles/sq. ft. of water surface.

It is the primary objective of this research to identify the immediate effects of aluminum-coated fiberglass chaff on typical estuarine species common to the Navy Research Laboratory Radar Testing Site, Chesapeake Beach, Maryland.

Methods

Rationale for Selection of Species

Species were chosen based on several criteria: 1) ecological abundance, 2) commercial importance, 3) presence at the test site, 4) availability and ease of maintenance in the laboratory, 5) feeding type.

The American oyster, Crassostrea virginica, is one of the most commercially important bivalve molluscs. The harvest value in 1975 approached \$46 million, with Maryland being the largest producer on the Atlantic Coast (NMFS, 1977). Oysters are typically estuarine and inhabit shallow waters extending intertidally to depths of 100 ft. The oyster is the primary species cultured at the University of Delaware's Mariculture Laboratory. For this reason, various life history stages were available for testing.

Mytilus edulis, the blue mussel, is a common bivalve mollusc in the Delaware region. Although under utilized, it is a commercially important species (Danforth, 1976). The mussel has a wide geographic range from the

Arctic Ocean to North Carolina and is found both intertidally and subtidally (Maurer, et al., 1976). Recently, Mytilus has received attention as a possible worldwide indicator organism for various heavy metal and hydrocarbon pollutants (Lee, 1972).

Hydroides (Eupomatus) dianthus is ecologically important because its calcareous tubes provide niches for other benthic invertebrates such as xanthid crabs, amphipods, and polychaetes. Large populations form reefs which are often important sport fishing sites. Hydroides are distributed widely with a geographic range extending from Massachusetts to the West Indies. They are found intertidally to depths of 30 m (Day, 1973). Hydroides can be obtained intertidally with little or no damage and can be maintained in the laboratory with minimal effort. As a filter feeding polychaete, they may be among the first to become exposed and susceptible to chaff in the environment.

The blue crab, Callinectes sapidus, was selected for the study since it is the most important commercial and ecologically dominant crustacean in the Western Atlantic (Costlow and Bookout, 1959). The blue crab is an omnivorous scavenger that is commonly found from Cape Cod to the Gulf of Mexico (Williams, 1965).

Several criteria were used to select candidate fish species for exposure to aluminum-coated fiberglass chaff. Fish which are of commercial, ecological, or sport fishing importance were considered. An attempt was made to include species which displayed different feeding types. Originally the menhaden (Brevoortia tyrannus), a plankton feeder; the bluefish (Pomatomus saltatrix), a pelagic carnivore; and the summer flounder (Paralichthys dentatus), a demersal carnivore; were selected for study.

The menhaden is taken commercially on the Atlantic and Gulf Coasts, and while it is not eaten by man, it is the most valuable commercial species taken in this country. Since it sieves the water to capture plankton, it is thought that it would likely ingest floating chaff. In late summer small menhaden were readily obtainable in the vicinity of the marine laboratory and were tested during the course of this study.

Summer flounder presented many problems as a test organism. Due to the late start of the project, fish were difficult to obtain and had to be collected in deep water by otter trawl. Although an adequate number of 30-50 cm sized flounders were collected, more than two-thirds of the fish did not survive the two-week acclimatization period. Survivors held for approximately a month in 4' x 8' fiberglass tanks refused a variety of food. The fish were transferred to larger (10' x 10') concrete tanks where they began to feed on live killifish, Fundulus heteroclitus. It took several weeks of acclimatization with food to determine how many bait fish the flounder would consume. Live killifish impacted with chaff were then fed to the flounder. As experimental feeding started, temperatures fell and feeding ceased. A further decrease in temperature resulted in mortalities which reduced numbers to a level where data would be of no statistical value. As a result, summer flounder were not tested in this study.

Bluefish proved equally problematical. The study was originally projected to begin in early summer. When the project finally started in September, small snapper bluefish were not available. Year old fish easily taken in seines in early summer had doubled in size presenting severe holding problems. Increased size and speed made seine avoidance the rule rather than the exception.

Due to the difficulties encountered with the above candidate species, the killifish, Fundulus heteroclitus, was used. The killifish or salt water minnow is common in shallow parts of the estuary, especially in tidal creeks. Fundulus is ecologically very important in the food web which includes most important sport fishes. It is an omnivore, consuming both plant and animal matter (Bigelow and Schroeder, 1953). Killifish are highly territorial with a "home range" of less than 50 ft. (Lotrich, personal communication). If exposed to chaff in an area, they would not likely avoid it by leaving the area as the more migratory bluefish might. Finally, Fundulus were readily available and adapted well to holding in the laboratory.

Experimental Procedures

The concentration or amount of aluminum fiberglass chaff added to the water was based on fallout occurring from actual field deployment of the material. It has been estimated that the fallout was approximately 53 dipoles/sq. ft. All organisms in these experiments were exposed to chaff at 100X and 1000X concentrations based on the surface area of the experimental chambers. High concentrations of chaff were used to simulate the most detrimental conditions and to assure that any negative biological effects were observed within the experimental period. Examination of the aluminum fiberglass chaff canisters showed that there are three lengths of chaff: 8.1 cm (long), 3.0 cm (medium), and 1.5 cm (short). Average weights determined by weighing 18 dipoles ten times are: .00197 g, .00098 g, and .00059 g, respectively. The above gramic weight equivalents became the standards for determining the amounts of chaff necessary in all tests.

In all experiments natural seawater from two sources, Indian River and Delaware Bay, were used. Oyster larvae and oyster feeding experiments performed at the University of Delaware's Mariculture facility utilized Indian River water. All finfish, crab, and filter feeder experiments were run using Delaware Bay water. In general, Indian River water had higher salinities and was less turbid than Delaware Bay water. Specific physical properties are presented in each experimental section.

Crassostrea virginica Larval Experiments

The two-liter beakers used as experimental chambers in the 48-hour larval bioassay had a surface area of .157 sq. ft. where a concentration of 8.32 dipoles would be considered to be a normal concentration of dipoles. At 100X and 1000X concentrations each beaker would have to contain 277 and 2,773 dipoles of each of the three sizes. The weights of each of the three sizes at 100X concentrations from long to short are: .03031 g, .01508 g, and .00907 g. At 1000X the weights are: .30348 g, .15097 g, and .5434 g, respectively. For pulverized chaff, aggregate weights of .05446 g and .5453 g were used for 100X and 1000X concentrations, respectively.

Oyster larvae were obtained by stripping female and male oysters as detailed in Loosanoff and Davis (1963). Tarzwell (1969) and Woelke (1972) detail procedures to follow in using 0 to 48 hour old oyster larvae as a bioassay technique. Three thousand fertilized eggs were placed in each control and experimental beaker along with 1.5l of 5 μ filtered natural Indian River seawater and chaff.

The water temperature was held between 24 and 28°C during the 48-hour experimental period. The beakers were aerated via Pasteur pipettes

at flow rates that averaged between 14 and 22 ml/sec. Oxygen values monitored with a YSI oxygen meter averaged 7 ppm. Salinity determined by refractometer was 28 o/oo. The long form chaff tended to clump and remain in suspension, while the pulverized chaff sank rapidly to the bottom.

Because the pulverized chaff was not a natural form, an attempt was made to characterize its physical properties. The chaff was measured for specific gravity, observed in various dispersant concentrations, run through settling tubes, dry sieved through 230 mesh sieve, and viewed through a microscope.

Specific gravity was determined using a Beckman Model 930 Air Comparison Pycnometer. A value of 2.59 (quartz standard 2.65) was determined. After determining the specific gravity, settling rates were predicted. However, clumping of particles precluded accurate determination of settling rates. The chaff settled as globular masses approximately 0.1-4.0 cc in size. The settling velocity of the clumps ranged from 0.01 to 0.12 m/sec in distilled water at 25°C, a rate approximating that of fine sand grains. The cross sectional diameter was less than 62 μ. The width to length ratio of the pulverized chaff was 1:10 (shape factor 0.1). The shape factor (0.1) is far below the limits generally accepted for natural sediments that are spherical (1.0) or disc (0.5). In summary, the pulverized chaff does not behave similarly to natural types of sediment.

After 48 hours the water was poured through a 37 μ screen to catch and concentrate the larvae. The concentrated larvae were washed into small beakers and diluted to a 30 ml volume. Three 1 ml samples from each beaker were counted and defined as normal, abnormal, undeveloped, and dead. Abnormal larvae have shell deformities such as scalloped edges in the D stage. Undeveloped larvae were defined as those larvae

that had not matured to the shelled D stage, but remained as soft, trochophore larvae.

A second experiment using 1 'ı, old oyster larvae was developed as previously detailed for the 0-4 ı .. larval bioassay. All chaff concentrations are identical to those used in t e previous 0-48 hour larval experiments. Larvae from the same stock us. in the 0-48 hour larval bioassay were maintained in large fiberglass cones and fed algae on a daily basis. Larval rearing techniques are further detailed in Loosanoff and Davis (1963). When the larvae were ten days old (pediveliger stage), 1,500 larvae were added to each 2 liter beaker (.157 sq. ft.) consisting of 1.5 liter seawater, algal food supplement and chaff. After 48 hours the water was poured through a screen to catch and concentrate larvae. In an identical procedure to the 0-48 hour larval experiment, larvae were counted, measured, and defined as normal, abnormal, and dead.

Physical properties such as salinity, D.O., and temperature were with the ranges previously reported for the 0-48 hour larval experiments.

Results and Discussion

The raw data for the 0-48 hour and 10-day old larval bioassays appear in Tables 1 and 2, respectively. Based on Table 1 the 0-48 hour old oyster larvae are clearly effected by the chaff's presence at both 100X and 1000X concentrations. There is approximately 20-30% survival in all controls, while mortalities are 100% in all experimental beakers. Due to the rapid decomposition of dead larvae, no larvae were found in any of the experimental groups.

There is some toxic effect associated with the chaff in this study. Baseline data on the mariculture system and the water used in these

Table 1

Raw data for 48-hour oyster larvae bioassay. Numbers represent actual larvae count from 1 ml sample.

Control

	Replicate 1			Replicate 2			Replicate 3			Replicate 4		
	1	2	3	1	2	3	1	2	3	1	2	3
Normal	27	24	18	28	22	17	22	20	37	41	38	35
Abnormal	2	6	8	2	3	2	0	0	1	2	0	0
Undeveloped	0	0	0	0	0	0	0	0	0	0	1	0
Dead	1	3	2	2	5	0	1	3	1	4	1	2

100X Long Form

	Replicate 1			Replicate 2			Replicate 3			Replicate 4		
	1	2	3	1	2	3	1	2	3	1	2	3
Normal	1	1	1	0	0	0	0	0	0	0	0	0
Abnormal	0	0	0	0	0	0	0	0	0	0	0	0
Undeveloped	0	0	0	0	0	0	0	0	0	0	0	0
Dead	0	0	0	0	0	0	0	0	0	0	0	0

1000X Long Form

	Replicate 1			Replicate 2			Replicate 3			Replicate 4		
	1	2	3	1	2	3	1	2	3	1	2	3
Normal	0	0	0	0	0	0	0	0	0	0	0	0
Abnormal	0	0	0	0	0	0	0	0	0	0	0	0
Undeveloped	0	0	0	0	0	0	0	0	0	0	0	0
Dead	0	0	0	0	0	0	0	0	0	0	0	0

Table 1 (continued)

Control

	Replicate 1			Replicate 2			Replicate 3			Replicate 4		
	1	2	3	1	2	3	1	2	3	1	2	3
Normal	54	238	168	14	17	9	23	25	24	19	17	9
Abnormal	10	25	23	0	3	0	1	1	1	2	0	0
Undeveloped	1	2	0	0	0	0	0	0	0	0	0	0
Dead	8	18	5	1	2	1	3	2	2	2	3	3

100X Pulverized

	Replicate 1			Replicate 2			Replicate 3			Replicate 4		
	1	2	3	1	2	3	1	2	3	1	2	3
Normal	0	0	0	0	1	0	0	0	0	0	0	0
Abnormal	0	0	0	0	0	0	0	0	0	0	0	0
Undeveloped	0	0	0	0	0	0	0	0	0	0	0	0
Dead	0	1	0	0	0	0	0	0	0	0	0	0

1000X Pulverized

	Replicate 1			Replicate 2			Replicate 3			Replicate 4		
	1	2	3	1	2	3	1	2	3	1	2	3
Normal	0	0	0	0	0	0	0	0	0	0	0	0
Abnormal	0	0	0	0	0	0	0	0	0	0	0	0
Undeveloped	0	0	0	0	0	0	0	0	0	0	0	0
Dead	0	0	0	0	0	0	0	0	0	0	0	0

experiments is detailed later in the report. However, handling of seawater through pumps, hoses, etc. increases the metal content above that normally encountered. It is also apparent that some heavy metals have leeched from the chaff as all experimental samples are higher in trace metal concentrations than the control (Table 19).

Data concerning the 10-day old oyster larvae (pediveliger stage), is detailed in Table 2. One-Way Analysis of Variance (Anova, F Test) was performed on group mean sizes and larval counts (Tables 3 and 4). The size of the larvae are significantly smaller in the 1000X long form than in the control. It is quite probable that the chaff had a detrimental effect on larval growth. There is no significant difference in sizes for the pulverized form. Healthy larvae spend the majority of their time in the water column and will not come in direct physical contact with the pulverized chaff which sinks rapidly. It is possible that both physical and chemical properties work synergistically to produce detrimental effects with older larvae.

Examination of larval density (Table 4) shows that the chaff has no negative effect on survival. In all replicates the mean larval density was greater in the 100X pulverized group than the control or 1000X pulverized group. There is no apparent biological reason to explain this anomaly. It is concluded that the chaff had no lethal effect on older larvae, but may have sublethal effects which are not easily assessed.

Crassostrea virginica Juvenile Feeding Experiments

The feeding response of bivalve molluscs has been widely studied. It is well known that various pollutants depress the feeding rates of oysters and clams. Epifanio and Srna (1975) report that ammonia concentrations of

Table 2

Raw data for one-week old oyster larvae bioassay. Numbers represent actual larvae count from 1 ml sample.

Control

	Replicate 1			Replicate 2			Replicate 3			Replicate 4		
	1	2	3	1	2	3	1	2	3	1	2	3
Normal	86	112	59	108	186	77	41	115	146	109	140	96
Abnormal	1	0	0	0	0	0	0	0	1	2	0	0
Dead	4	11	2	7	15	7	3	23	20	1	15	10

100X Long Form

	Replicate 1			Replicate 2			Replicate 3			Replicate 4		
	1	2	3	1	2	3	1	2	3	1	2	3
Normal	97	231	160	137	72	93	97	100	183	68	47	130
Abnormal	0	0	0	0	0	0	1	0	0	0	0	0
Dead	15	16	18	9	11	10	16	4	30	4	1	3

1000X Long Form

	Replicate 1			Replicate 2			Replicate 3			Replicate 4		
	1	2	3	1	2	3	1	2	3	1	2	3
Normal	116	121	144	108	77	110	131	120	90	38	67	52
Abnormal	0	0	0	2	0	0	4	1	0	2	0	0
Dead	7	3	4	2	0	0	8	5	0	5	1	0

Table 2 (continued)

Control

	Replicate 1			Replicate 2			Replicate 3			Replicate 4		
	1	2	3	1	2	3	1	2	3	1	2	3
Normal	71	114	77	85	101	120	50	66	54	148	94	124
Abnormal	0	1	0	0	1	3	0	3	0	0	0	0
Dead	6	2	4	17	7	7	4	0	3	13	0	1

100X Pulverized

	Replicate 1			Replicate 2			Replicate 3			Replicate 4		
	1	2	3	1	2	3	1	2	3	1	2	3
Normal	124	114	183	108	221	191	86	227	171	118	141	216
Abnormal	0	0	0	0	0	0	1	0	0	2	1	0
Dead	10	6	6	14	6	5	11	7	2	6	5	4

1000X Pulverized

	Replicate 1			Replicate 2			Replicate 3			Replicate 4		
	1	2	3	1	2	3	1	2	3	1	2	3
Normal	121	103	79	77	56	106	74	80	90	115	109	97
Abnormal	0	0	0	0	0	0	6	3	2	1	1	1
Dead	0	3	0	3	2	4	3	4	5	3	5	4

Table 3

Mean larval sizes for 10-day old larvae in microns based on 20 measurements per group after 48 hours exposure to chaff.

Control	100X Long Form	1000X Long Form
195.0	187.3	177.6
194.9	175.7	174.4
208.5	191.2	176.9
192.5	186.2	173.5

F = 14.68*

T_c vs. 100X = 3.845*

T_c vs. 1000X = 6.868*

Control	100X Pulverized	1000X Pulverized
177.2	187.2	185.4
188.5	160.8	181.4
191.8	195.4	183.2
191.9	205.9	191.2

F = .0384

* Denotes significant value at F_{05} or 95% confidence level.

Table 4

Three-count mean larval densities for 10-day old larvae after 48 hours of exposure to aluminum fiberglass coated chaff.

Control	100X Long Form	1000X Long Form
86	162	127
124	102	98
101	125	114
115	82	52

$F = .4761$

Control	100X Pulverized	1000X Pulverized
87	161	101
102	140	80
57	173	81
122	156	107

$F = 15.03$*

T_c vs. 100X = 3.432*

T_c vs. 1000X = 0.0225

*Denotes significant F value at F_{05} or 95% confidence level.

7.2 ppm were considered sublethally toxic, as evidenced by decreased filtering efficiency. Keck, et al. (1977) reported that the WSF of Nigerian crude oil decreased filtering rates at concentrations as low as 0.6 ppm. Loosanoff and Tommers (1948) found that 0.1 g/l of particulate matter such as kaolin or Fuller's earth depressed filtration rate by 50%. Similarly, Rice and Smith (1958) showed that 5 g/l of silt lowered the filtration rate of M. mercenaria. It is probable that chaff could lower filtration by physical, particulate, or chemical toxic action on the oysters.

Procedures for bivalve feeding studies are detailed in Epifanio and Srna (1975) and Keck, et al. (1977). Two liter glass beakers (surface area .157 sq. ft.) were used as experimental chambers for two sets of experiments. Chaff concentrations were identical to those used in larval experiments: .03031 g (long), .01508 g (medium), and .0.907 g (small) for 100X and .30348 g, .15097 g and .5434 g, respectively at 1000X concentrations.

Ten juvenile oysters between 1.7 and 2.4 cm were scrubbed in dilute Chlorox water and placed in each beaker. One liter of 0.25 micron filtered seawater, 800 ml of algae culture, and the desired amount of chaff were added to each beaker. Thalassiosira sp. was exclusively used as a food source during the experiments. Algal counts were made by taking 2 ml of sample and diluting with 98 ml of millipore filtered seawater. This sample was then counted using Model ZB Coulter Counter with 1/4 ampere and .088 aperture current settings. The use of discrete settings assures that only the desired species of algae is counted by the Coulter Counter. By millipore filtering the water bacteria and undesirable algal species are removed, lowering background counts.

Algal counts were made immediately after the 800 ml of algae was added to the beakers and in Group I experiments after 24 hours, and in

Group II experiments after 4 hours. Throughout the duration of the experiments seawater changes and algal food supplements were made daily, with an exception during the Yule holiday season when changes were made every other day.

A third group of experiments was performed with 40 oysters (10 from each of 4 replicates) consolidated in ten-gallon aquaria (1 sq. ft. surface area). In these experiments, 10 l of filtered seawater, 20 l of algal food supplement, and chaff were added to each aquaria. Initial algal counts were made immediately and after 24 hours. The weights of chaff used in the aquaria studies were: .1212 g (long), .0603 g (medium), and .0362 g (short) for 100X and 1.212 g, .6032 g and .3628, respectively for 1000X. For pulverized chaff aggregate weights of .218 g and 2.181 g were used for 100X and 1000X concentrations respectively.

With the exception of temperature, physical and chemical parameters in the feeding studies closely approximated those of the larval studies. Salinities ranged from 27 to 30 o/oo; aeration rates averaged 18 ml/sec., and D.O.'s were all above 6 ppm. Temperatures were considerably lower ranging between 16.5°C and 20°C, mean 18.5°C. Although 20°C is considered an optimum temperature for the growth and survival of adult oysters, temperatures during these experiments are within accepted limits for active feeding and growth of oysters.

The results from each experiment are presented as means of three counts for each replicate in Tables 5 through 9. In 16 experiments only 3 showed statistically significant differences between controls and experimental treatments, with less algae being consumed by oysters exposed to chaff. Pooled data representing averages from all similar experiments showed no significant effects of chaff on the feeding rates of the oysters (Table 8).

Examination of Table 9 indicates that oysters in the controls grew faster than those exposed to 1000X concentrations of chaff. However, with the exception of the 100X pulverized value, all t values, comparing mean sizes before and after exposure, are insignificant.

Although there was no statistically significant difference between controls and test organisms in regard to uptake of algae, certain trends emerged. The ingestion of algae cannot be directly equated with growth. When an organism is stressed, a great deal of energy is expended on regulation of physiological processes. As a result, growth rates are generally lower for perturbed organisms. It is anticipated that by extending the experimental period, observed differences in growth would increase, probably resulting in statistically significant differences.

The feeding experiments would be improved by using a flow through recycling system which would provide more natural conditions. It would also be necessary to study the physical and chemical properties of chaff to determine at what concentration the material is most toxic. For example, chaff could be most toxic immediately after fallout or it might require considerable aging before toxic materials are released.

Table 5

Group I: Final algal counts in cells/ml after 24 hours by test oysters, *Crassostrea virginica*

Long Form:

Experiment I   Starting Density: 3.153×10^5

Control	100X	1000X
$.236 \times 10^5$	.205	.551
.220	.218	.387
.191	.152	.249
.180	.264	.521

F = 8.72*

T_c vs. 100X = 0.1065

T_c vs. 1000X = 3.322*

Experiment II   Starting Density: 3.734×10^5

Control	100X	1000X
$.216 \times 10^5$	.129	.169
.131	.270	.183
.287	.183	.179
.154	.191	.204

F = .0659

Experiment III   Starting Density: 1.581×10^6

Control	100X	1000X
$.256 \times 10^5$	.181	.219
.241	.182	.166
.181	.291	.268
.295	.196	.253

F = .4021

Table 5 (continued)

Pulverized:

Experiment I   Starting Density: 3.153×10^5

Control	100X	1000X
$.436 \times 10^5$	.332	.214
.297	.290	.354
.447	.341	.188
.282	.296	.285

F = 2.375

Experiment II   Starting Density: 3.734×10^5

Control	100X	1000X
$.144 \times 10^5$	.266	.322
.183	.161	.282
.306	.066	.234
.136	.077	.189

F = 2.177

Experiment III   Starting Density: 1.581×10^6

Control	100X	1000X
$.225 \times 10^5$	.126	.308
.251	.297	.297
.208	.160	.279
.255	.269	.348

F = 4.136*

T_c vs. 100X = 0.6732

T_c vs. 1000X = 7.227*

*Denotes significant F value at F_{05} or 95% confidence level.

Table 6

Group II: Final algal counts in cells/ml after 4 hours of feeding by test oysters, *Crassostrea virginica*

Long Form:

Experiment I   Starting Density: 1.700×10^6

Control	100X	1000X
7.613×10^5	10.617	14.110
10.135	12.384	10.151
12.664	6.796	13.089
9.972	15.223	12.164

F = .8002

Experiment II   Starting Density: 1.410×10^6

Control	100X	1000X
1.554×10^5	1.488	1.499
1.660	1.634	1.253
1.576	.854	1.422
1.293	1.299	1.250

F = .8917

Experiment III   Starting Density: 1.407×10^6

Control	100X	1000X
$.773 \times 10^5$	2.856	2.322
.967	1.856	6.280
1.891	2.129	5.748
.782	3.676	2.627

F = 5.707*

T_c vs. 100X = 2.565

T_c vs. 1000X = 3.540*

Experiment IV   Starting Density: 1.439×10^6

Control	100X	1000X
3.928×10^5	2.889	4.284
4.575	2.782	5.938
2.574	3.935	5.968
.942	4.071	2.187

F = 1.307

Table 6 (continued)

Pulverized:

Experiment I Starting Density: 1.700×10^6

Control	100X	1000X
9.674×10^5	11.572	10.540
6.516	7.789	7.688
4.161	13.343	9.538
6.336	9.852	7.725

$F = 3.711$

Experiment II Starting Density: 1.410×10^6

Control	100X	1000X
1.179×10^5	.652	.495
1.476	.527	.622
.885	1.112	.882
.727	1.063	.290

$F = 2.868$

Experiment III Starting Density: 1.407×10^6

Control	100X	1000X
2.383×10^5	.194	3.719
4.836	2.581	1.711
2.504	2.388	5.378
4.275	6.692	1.326

$F = .0818$

Experiment IV Starting Density: 1.439×10^6

Control	100X	1000X
3.238×10^5	4.300	2.147
4.087	3.335	2.676
6.705	4.082	1.101
2.602	4.452	1.219

$F = 5.274$*

T_c vs. 100X = 0.0176

T_c vs. 1000X = 2.426

*Denotes significant F value at F_{05} or 95% confidence level.

Table 7

Group III: Aquaria experiments. Final algal counts in cells/ml after 24 hours of feeding by test oysters, *Crassostrea virginica*.

Long Form:

Starting Density: 1.303×10^6

Control	100X	1000X
$.259 \times 10^5$	.297	.549
.607	.324	.694
1.65	5.139	.709

F = .5085

Pulverized:

Starting Density: 1.303×10^6

Control	100X	1000X
1.423×10^5	1.247	1.730
.629	.774	1.667
4.402	3.806	5.522

F = .2325

Table 8

Pooled final algal cell counts in cells/ml.

Long Form:

Group I Experiments (24 hours)

Control	100X	1000X
$.2070 \times 10^5$	.209	.427
.197	.193	.184
.243	.213	.227

F = .8301

Group II Experiments (4 hours)

Control	100X	1000X
1.009×10^5	1.125	1.238
1.520	1.318	1.356
1.103	2.629	4.244
3.005	3.419	4.594

F = .8241

Pulverized:

Group I Experiments (24 hours)

Control	100X	1000X
$.365 \times 10^5$	.315	.260
.192	.142	.256
.235	.213	.308

F = .3999

Group II Experiments (4 hours)

Control	100X	1000X
$.667 \times 10^5$	1.064	.887
1.067	.839	.572
3.499	2.964	3.034
4.158	4.042	1.786

F = .3181

Table 9

Mean growth of oysters determined by subtracting the before and after group means, based on 40 measurements/group. Length expressed in cm. T values compare mean sizes before and after exposure to chaff.

	Control	100X	1000X
Long Form:			
	.07 cm	.05 cm	.03 cm
	T = 1.367	T = 1.201	T = 0.747
Pulverized:			
	.07 cm	.10 cm	.03 cm
	T = 1.814	T = 2.321*	T = 1.474

*Denotes significant T value at 95% confidence level.

Mytilus edulis and *Hydroides dianthus* Experiments

Methods

Nine 55-gallon (208 liter) aquaria were employed, each containing 32 *Mytilus edulis* and approximately 32 *Hydroides dianthus*. Since *Hydroides* occurs in clusters, one cannot break off an exact number for each aquarium. All the individuals were acclimated for a 5-day period and were fed a diet of phytoplankton consisting of *Isochrysis galbana parke* and *Thalassiosira pseudonana* Clone 3-H twice weekly throughout the experiment.

After the acclimatization period, three aquaria apiece were established for 100X, 1000X, and controls. Since the chaff was reported to normally be found in concentrations of 53 dipoles per square foot (0.09 m^2), the weight of 53 dipoles of chaff was used as the basis for the calculations shown in Table 10.

The chaff was added to the water. Since it had a tendency to cluster into bundles, an attempt was made to separate it into individual strands as much as possible. The water was then agitated with a stirring rod once a day to get the chaff in suspension, as once the surface tension was broken it readily sank to the bottom of the aquaria. Two air stones in each tank were used to supply sufficient oxygen for the animals, as they were kept in a static system. These also helped in maintaining the chaff in suspension for a greater period of time.

Results and Discussion

Based on Table 11, the 1000X concentration was lethal to the polychaete, *Hydroides dianthus*. By the fifth day of the experiment there was complete mortality at this concentration. In the 100X concentration mortalities occurred at a much slower rate. The *Hydroides* did not show any mortality

until the 11th day, after which there were only a few deaths (maximum 7) each day. However, by the 21st day, the second replicate had complete mortality, and replicate one had 53% mortality. Although there was no mortality in replicate three among Hydroides, there was some mortality in the 100X replicate for Mytilus.

In all cases except the control the Hydroides reaction time to an external stimulus (shadows or tapping on the sides of the tank) was noticeably decreased after the third day, the reaction being the withdrawal into the tubes. Although this was not quantified by measuring actual time differences among the various concentrations throughout the experiment, it is believed that this may be an indication of a sublethal effect.

A one-way Analysis of Variance (Anova) was run to determine whether there were any substantial differences among the mortality rates of H. dianthus exposed to three concentrations of chaff. The Anova showed a significant difference at the 95% confidence interval. An F value of 5.1423 with 2 and 6 degrees of freedom was required to be significant at the 95% confidence interval making the computed value of this data 5.4174 significant. A t-test was then computed to determine at which concentration this difference occurred. Again at the 95% confidence interval there was found to be a significant difference between the 1000X vs. control ($t = 5.119$), while the difference between the 1000X vs. 100X ($t = 1.920$) and 100X vs. control ($t = 1.7492$) showed no significant difference in mortality. A t value of 2.776 with four degrees of freedom was necessary to be significant at the 95% confidence interval.

It can be seen (Table 12) that greater than 50% mortality occurred in the 100X concentration, thus suggesting that there should be a significant difference between 100X and the control. However, the use of statistics

may be restrictive here due to the small sample size, especially when one or more of these values are zero.

Based on the present data it is difficult to statistically prove a difference between the control and 100X. However, we submit that future studies should increase the numbers of individuals and replicates over a longer period of time to insure proper statistical inferences. Moreover, there is also a need to determine the concentration where the lethal and sublethal effects are delineated.

Mytilus edulis were apparently unaffected by the chaff having only four individuals die in all the replicates and one of those in the control. The wet weights (Table 13) show that there was no significant change throughout the experiment. In future studies *M. edulis* should be exposed to pulverized chaff over an extended period of time. Temperatures in these experiments ranged between 16.0°C and 23°C, mean 18.9°C. Salinities and dissolved oxygen values averaged 28.4 o/oo and 8.2 ppm, respectively. These parameters were well within the tolerance range for the species tested (Gonzalez and Yevich, 1976; Castagna and Chanley, 1973).

Table 10

Calculation of chaff needed for the 100X and 1000X concentrations.

Area of aquaria: 1' x 4' or 4 square feet

$$\frac{1 \text{ sq. ft.}}{53 \text{ dipoles}} = \frac{4 \text{ sq. ft.}}{X \text{ dipoles}} \quad X = 212 \text{ dipoles}$$

18 = 1/3 of 53 dipoles
0.0197g = weight of 18 large size chaff
0.0098g = weight of 18 medium size chaff
0.00059g = weight of 18 small size chaff

100X

2120 dipoles needed

$$\frac{2120}{3} = 707 \text{ dipoles of each of the 3 sizes}$$

Large

$$\frac{18}{0.0197g} = \frac{707}{X}$$

$18X = 13.927$
$X = .773g$

Medium

$$\frac{18}{0.0098g} = \frac{707}{X}$$

$18X = 6.928$
$X = 0.385g$

Small

$$\frac{18}{0.00059g} = \frac{707}{X}$$

$18X = 0.417$
$X = 0.023g$

1000X

21,200 dipoles needed

$$\frac{21{,}200}{3} = 7067 \text{ dipoles of each of the 3 sizes}$$

Large

$$\frac{18}{0.0197g} = \frac{7067}{X}$$

$18X = 139.22$
$X = 7.73$

Medium

$$\frac{18}{0.0098g} = \frac{7067}{X}$$

$18X = 69.26$
$X = 3.85$

Small

$$\frac{18}{0.00059g} = \frac{7067}{X}$$

$18X = 4.17$
$X = 0.232$

Table 11

Daily mortality counts of *Mytilus edulis* and *Hydroides dianthus*.

Day of Experiment		Replicate 1 Control	Replicate 1 100X	Replicate 1 1000X	Replicate 2 Control	Replicate 2 100X	Replicate 2 1000X	Replicate 3 Control	Replicate 3 100X	Replicate 3 1000X
0	M*	0	0	0	0	0	0	0	0	0
	H*	0	0	0	0	0	0	0	0	0
1	M	0	0	0	0	0	0	0	0	0
	H	0	0	0	0	0	0	0	0	0
2	M	0	0	0	0	0	0	0	0	0
	H	0	0	2	0	0	2	0	0	2
3	M	0	0	0	0	0	0	0	0	0
	H	0	0	30	0	0	8	0	0	37
4	M	0	0	0	0	0	0	0	0	0
	H	0	0	-	0	0	9	0	0	-
5	M	0	0	0	0	0	0	0	0	0
	H	0	0	-	0	0	-	0	0	-
6	M	0	0	0	0	0	0	0	0	0
	H	0	0	-	0	0	-	0	0	-
7	M	0	0	0	0	0	0	0	0	0
	H	0	0	-	0	0	-	0	0	-
8	M	0	0	0	1	0	0	0	0	0
	H	0	0	-	0	0	-	0	0	-
9	M	0	0	0	0	0	0	0	1	1
	H	0	0	-	0	0	-	0	0	-
10	M	0	0	0	0	0	0	0	0	0
	H	0	0	-	0	1	-	0	0	-

*M = *Mytilus edulis*, H = *Hydroides dianthus*

Table 11 (continued)

Day of Experiment		Replicate 1			Replicate 2			Replicate 3		
		Control	100X	1000X	Control	100X	1000X	Control	100X	1000X
11	M	0	0	0	0	0	0	0	0	0
	H	0	0	-	0	4	-	0	0	-
12	M	0	0	0	0	0	0	0	0	0
	H	0	4	-	0	1	-	0	0	-
13	M	0	0	0	0	0	0	0	0	0
	H	0	2	-	0	2	-	0	0	-
14	M	0	0	0	0	0	0	0	0	1
	H	0	2	-	0	2	-	0	0	-
15	M	0	0	0	0	0	0	0	0	0
	H	0	2	-	0	0	-	0	0	-
16	M	0	0	0	0	0	0	0	0	0
	H	0	0	-	0	7	-	0	0	-
17	M	0	0	0	0	0	0	0	1	0
	H	0	5	-	0	6	-	0	0	-
18	M	0	0	0	0	0	0	0	0	0
	H	0	0	-	0	0	-	0	0	-
19	M	0	0	0	0	0	0	0	0	0
	H	0	0	-	0	2	-	0	0	-
20	M	0	0	0	0	0	0	0	0	0
	H	0	0	-	0	3	-	0	0	-
21	M	0	0	0	0	0	0	0	0	0
	H	0	2	-	0	5	-	0	0	-
Mortality Total	M	0	0	0	1	0	0	0	1	2
	H	0	17	32	0	33	19	0	0	39

Table 12

Total mortality data for *Hydroides dianthus*.

	Replicates					
	1		2		3	
Concentration	No. Dead	% Dead	No. Dead	% Dead	No. Dead	% Dead
Control	0	0	0	0	0	0
100X	17	53	33	100	0	0
1000X	32	100	19	100	39	100

Table 13

Wet weights of *Mytilus edulis*.

	Wet Weight (grams)	
Concentration	Day 0	Day 21
Replicate 1		
100X	173.3	173.4
1000X	213.8	213.7
	182.1	187.0
Replicate 2		
Control	162.3	160.1 (1 dead)
100X	193.1	195.7
1000X	148.4	148.7
Replicate 3		
Control	143.2	158.8
100X	183.0	176.0 (1 dead)
1000X	164.5	158.8 (2 dead)

Callinectes sapidus Experiments

Methods

Three tanks were employed. Each tank was 360 x 60 x 24 cm. All tanks contained six rubberized 50 x 43 cm wire trays which were equally spaced throughout the larger tank to provide for sufficient air and water circulation. These rubberized trays were further subdivided into four equal compartments with plastic 3.5 cm stretch mesh netting. This was done so that the feeding of each crab could be recorded separately and to avoid cannibalism. Three air stones were staggered throughout each tank to provide air and water circulation. The water in all tanks was emptied and refilled biweekly to prevent stagnation of the tanks. The water temperature was measured at each change to insure the water remained within ±1°. The water was allowed to settle first in a standing tank to reduce siltation. It was then pumped through a heat exchanger at the desired temperature into the crab tank. All the crabs were acclimated for a 5-day period. During the acclimatization period it was determined from feeding various amounts of food that a 6 gram (±1 gram) per crab block of fish gel was optimum for daily feeding during the experimental period.

After the acclimatization period, one tank each (each tank contained 24 crabs) was provided with 100X, 1000X concentrations, and control. The chaff was fed over a 21-day period so that by the 21st day the crabs had been exposed to the appropriate concentration.

The weight of 53 dipoles of chaff was used as the basis for the calculations in Table 14 since the chaff was reported to normally be found in concentrations of 53 dipoles per square foot (0.09 m^2). Only the small dipoles were used in mixing with food gel.

Each tank was cleaned biweekly, at which time salinity, dissolved oxygen, and temperature were recorded. Moreover, the remaining food was collected and a wet weight was taken.

Results and Discussion

The blue crab, Callinectes sapidus, had two mortalities, one in the 1000X on the 11th day and one in the control on the 12th day. They were preserved for further histological examination. There was no indication of growth (no molting) over the experimental period. The size and sex of each crab can be found in Table 15.

Two anomalies occurred in the data (Table 16). On the 12th and 20th days of collection control crabs were accidentally fed fish gel which had been frozen and thawed before feeding. The 100X and 1000X crabs always received fresh, unfrozen food gel. Because of these irregularities there is no statistical difference in the F value comparing food rejection. However, a t-test performed by pairing means results in a significance value of 2.574 at the 95% confidence level. Thus it can be said that the crabs are avoiding the food containing chaff.

Chaff was found in the crab feces in both the 100X and 1000X concentrations. However, the blue crab appeared to be capable of sorting large quantities (1000X) of chaff from its food source without causing any apparent short-term effects. The crabs in the 1000X concentration (Table 16) showed the highest reduction of food ingestion. This may indicate that they were approaching their tolerance level to the chaff. The dissolved oxygen, salinity, and temperature values averaged 9.02 ppm, 27.5 o/oo, and 15.1°C, respectively, and were well within the tolerance range for the blue crab (Tagatz, 1969).

It is suggested that if a second experiment is started that it be conducted during the crabs' natural seasonal period of scavaging activity, thus enhancing the success of the laboratory acclimatization period.

Table 14

Food gel formula.

Food Gel Formula

800 ml = H_2O

15 g = Agar

<u>175</u> g = Fish

900 g = Total Weight of One Tray

Grams of Chaff Needed for 100X and 1000X Concentrations

0.00177 grams = Weight of 53 Small Dipoles

<u>100X</u>	<u>1000X</u>
0.177 grams = Weight of 5,300 Small Dipoles	1.77 grams = Weight of 53,000 Small Dipoles
0.00843 g/day/crab = Total Weight of Dipoles Needed for 21-Day Experiment	0.0843 = Total Weight of Dipoles Needed for 21-Day Experiment

Each 6 gram block contained 0.00843 g for 100X and 0.0843 g for 1000X. Since each of the 24 crabs were to be fed one 6 gram block every day for 21 days, there was a total of 504 blocks or three trays of fish gel. Therefore, 504 X 0.00843 or 4.25 g total or 1.42 g for each tray for 100X and 504 X .0843 or 42.5 g total or 14.16 g for each tray for 1000X.

Table 15

Size and sex (point to point) of the blue crab, *Callinectes sapidus*.

Control Sex	Control Size (mm)	100X Sex	100X Size (mm)	1000X Sex	1000X Size (mm)
Male	144	Male	146	Male	164
	133		142		149
	143		136		141
	171		133		122
	141		130		110
	143		126		162
	125		148		132
	172		112		152
	143		136		124
	144		114		146
	150	Female	154		122
	137		147		128
	137		168		112
	157		146		135
	112		148	Female	139
	147		140		130
Female	146		151		144
	145		157		149
	142		134		152
	160		145		151
	144		141		148
	152		140		151
	139		117		155
	146		144		140
Average Males	143	Average Males	132	Average Males	136
Average Females	146	Average Females	154	Average Females	146

Table 16

Grams and percent of food gel not eaten by the blue crab.

Method 1

	Total Grams Fed	Days (Inclusive)	Control		100X		1000X	
	576	0- 3	9.90	1.7%	1.30	0.2%	8.70	1.5%
	432	4- 6	1.40	0.3%	5.25	1.2%	11.80	2.7%
	288	7- 8	5.50	1.9%	11.25	3.9%	43.85	15.2%
	576	9-12	110.80*	19.2%	68.65	11.9%	160.30	27.8%
	432	13-15	55.00	12.7%	57.50	13.3%	92.60	21.4%
	432	16-18	60.70	14.1%	96.40	22.3%	156.50	36.2%
	288	19-20	131.00*	45.5%	72.70	25.2%	132.60	46.0%
Totals	3,024		374.30		317.55		606.35	
Mean Percent				12.4%		10.5%		20.1%

T_c vs. 100X = 0.7295

T_c vs. 1000X = 2.574*

*food was frozen before feeding

Finfish Experiments

Methods

Brevoortia tyrannus

Juvenile menhaden 8-15 cm in fork length were collected during the late summer (1976) by cast net. Schools were located in the Broadkill River and approached by small boat. The cast net was thrown and captured fish transferred directly to 20-gallon containers. These were transported quickly to the laboratory. Handling was minimized and transfers were made quickly and carefully to avoid handling mortalities which approached 100%. This species is very delicate. Menhaden were held in running water in 4' x 8' fiberglass tanks to acclimatize to laboratory conditions. Running water was necessary to insure adequate planktonic food supply. A two-week acclimatization period was used. Temperature, salinity, and dissolved oxygen were monitored periodically. Since these animals were easily killed by handling, a small subsample was taken for initial fork length and weight measurements at the onset of the experimental period. Chaff of various lengths, in quantities equal to 100X and 1000X the average incident surface value were added to tanks of 27 menhaden.

The experimental tanks had 32 sq. ft. of surface area where a dipole concentration of 1,696 would be considered normal based on the 53/sq. ft. figure. Using the dipole weight factors detailed previously in the report, 6.18 g (long), 3.08 g (medium), and 1.85 g (small) dipoles were used for 100X concentrations. In 1000X tanks 61.8 g, 30.8 g, and 18.5 g of chaff were used respectively.

Fundulus heteroclitus

Killifish were collected using baited minnow pots from drainage ditches adjacent to Canary Creek, a tidal creek in the Broadkill River drainage basin. These were transferred to small buckets. Unlike the fragile menhaden, the killifish is extremely tolerant to wide ranges of temperature, salinity, and dissolved oxygen concentrations. Survival was 100% during most collection and handling. Fundulus were placed in 55-gallon (208 liter) aquaria and allowed to acclimatize for several weeks. Since chaff was to be fed to them incorporated within an artificial food, rather then directly in the water, they were fed a variety of foods, including the artificial food during the acclimatization period. The artificial "minnow chow" was made using the following recipe:

to 4 liters of water
add 875 g of dry dog food--allow to soften--stir occasionally
heat to boiling with stirring
add 75 g of nutrient agar--continue boiling 5 minutes
pour into three flat pans

The agar caused this mixture to gel while cooling. Cubes could then be cut and these did not lose their integrity in salt water. The Fundulus did not show a preference for other foods, so the minnow chow was considered acceptable. Three replicate experiments were run. To reduce the variability in the size of the killifish used, they were divided roughly into large, medium, and small fish. Each group had a control tank where the chow contained no chaff. For experimental animals to be exposed to chaff, appropriate amounts of chaff were stirred into the chow and a known volume of food (and therefore, a known amount of chaff) were fed to the fish each day.

The experimental aquaria had a 4 sq. ft. surface area with a normal concentration of 212 dipoles based on the 53 sq. ft. average. Using the dipole weight factor previously determined for small chaff (.00059 g/18 dipoles), a weight of .694 g for 100X and 6.94 g for 1000X was the weight of chaff to be fed during the 21-day period. Because the different size groups of killifish consumed different amounts of food, the time frame was variable to insure exposure to similar amounts of chaff-tainted food. Large fish were exposed for three weeks, medium fish for four weeks, and small fish for five weeks. During this period, water was changed every 5-7 days and aerated between changes. Because of their hardiness, Fundulus could be weighed and measured before and after the end of the experimental period. Changes in size over time and differences between groups were determined statistically using a t-test.

Results and Discussion

Table 17 shows a summary of size means and mortalities for various menhaden treatment groups. Mortality is rather random in pattern. Control animals showed 7.4% mortality. Those exposed to 100X chaff concentrations showed 33% mortality, but menhaden exposed to maximum 1000X concentration showed 0% mortality. The relationship between mortality of menhaden and chaff is inconclusive based on these results. Comparisons (t-test) between means of length and weight showed only one significant difference. There was a statistically significant difference in live weight at the F_{05} level between the control and 1000X groups. Although there is a greater difference between 1000X and the initial subsample in weight, this difference was not significant due to small sample size and high variability of the subsample. Due to the fact

that before and after weighing was not possible, this weight difference may be due to chance or it may be due to a reduction in plankton populations upon which the menhaden feed. This possibility is given added credibility in light of the effects of chaff on planktonic oyster larvae presented elsewhere in this report. If avoidance of chaff were responsible for the lower weight, this would be expected to eventually result in mortalities with an increased experimental period.

Table 18 shows a summary of Fundulus data. Since there were rather large size differences between replicates, the data was not pooled as was done with the menhaden. Mortality trends were apparently unrelated to chaff concentrations. Large and medium fish showed no mortalities during the three and four week experimental periods respectively. Small fish seemed to be more delicate. Control and 1000X groups showed 3.17% mortalities, while the intermediate 100X group showed 15.6% mortality. Mortalities were not related to water quality since monitoring showed salinity, temperature, and dissolved oxygen to be well within the range described for this species (Bigelow and Schroeder, 1953). Some mortalities of small Fundulus were observed during handling and tank cleaning and this appears to be the cause of most losses. Statistical comparison by t-test of initial and final lengths and weights show that while almost all groups showed some growth, in no case was the change statistically significant at the 0.05 level during the experimental period. All small fish showed similar small increases in length and weight regardless of treatment. Medium fish showed some differences in pattern, but these were apparently unrelated to the chaff exposure. Control fish showed almost no change in size and fish fed 1000X chaff concentrations showed the only weight loss shown by any group. The

Table 17

Menhaden data summary.

	Mean Fork Length	Mean Live Weight
Initial Subsample	103.8 mm (st. dev. 0.745)	17.8 g (st. dev. 4.23)
Control	107.4 mm (st. dev. 0.486) T_{ss} = 1.723	17.6 g (st. dev. 2.551) T_{ss} = .4954
100X Chaff	107.8 mm (st. dev. 0.514) T_c = 1.696	17.19 g (st. dev. 2.80) T_c = 1.673
1000X Chaff	104.2 mm (st. dev. 0.690) T_c = 1.45	15.65 g (st. dev. 3.168) T_c = 2.43*

( ) denotes standard deviation

* Denotes significant T value at 95% confidence level.

	Mortality
Control	7.4%
100X Chaff	33.0%
1000X Chaff	0.0%

T_{ss} = T value for subsample vs. control

T_c = T value for control vs. experimental value

Table 18

Fundulus data summary.

	Mean Initial Length	Mean Final Length	Mean Δ Length
Controls:			
Large	67.88 mm (13.26)	68.24 mm (12.30)	+ 0.36 mm
Medium	65.69 mm (7.92)	65.78 mm (8.53)	+ 0.09 mm
Small	57.03 mm (5.17)	58.10 (4.87)	+ 1.07 mm
100X Chaff			
Large	71.25 mm (11.49)	71.00 mm (11.83)	- 0.25 mm
Medium	67.91 mm (6.41)	68.91 (6.47)	+ 1.00 mm
Small	49.13 (2.89)	50.00 (2.97)	+ 0.87 mm
1000X Chaff			
Large	69.78 mm (10.15)	70.06 (10.25)	+ 0.28 mm
Medium	65.59 (4.99)	66.03 mm (4.72)	+ 0.44 mm
Small	56.97 mm (5.62)	57.84 (5.53)	+ 0.87 mm

Table 18 (continued)

Controls:

	Mean Initial Weight	Mean Final Length	Mean Δ Weight
Large	4.47 g (2.70)	4.84 g (2.81)	+ 0.37 g
Medium	4.009 (1.684)	4.025 (1.671)	+ 0.014 g
Small	2.38 g (0.660)	2.49 g (0.678)	+ 0.11 g
100X Chaff			
Large	5.55 g (3.13)	6.07 g (3.47)	+ 0.52 g
Medium	4.24 g (1.43)	4.48 g (1.56)	+ 0.24 g
Small	1.32 g (0.379)	1.52 g (0.354)	+ 0.20 g
1000X Chaff			
Large	5.044 (3.038)	5.369 (3.69)	+ 0.325 g
Medium	3.819 (0.960)	3.644 (0.767)	- 0.175 g
Small	2.27 g (0.839)	2.384 (0.755)	+ 0.114 g

Mortality

	Control	100X Chaff	1000X Chaff
Large	0%	0%	0%
Medium	0%	0%	0%
Small	3.1%	15.6%	3.1%

( ) denotes standard deviation

intermediate 100X chaff group, however, had the largest growth increment shown by any treatment group. Large fish all showed similar small increases in weight and less growth in length than other size groups. This is to be expected since weight gain per growth interval increases greatly among fish of this size. Large fish fed 100X chaff concentrations showed the only negative change in body length (-.025 mm). This decline is probably not real, but represents the level of precision of measurement. Since no treatment group showed any significant change in total length or live weight during the experimental period, it would seem that the experimental period should be increased until significant growth changes can be measured. At that time, if all groups show significant length and weight gain, then it can be stated that chaff is not detrimental to growth of Fundulus. Conversely, if groups fed chaff declined significantly in weight or failed to increase in length while control groups did grow, then chaff might be cited as being detrimental to Fundulus growth. In addition to increasing the experimental period, it would seem to be worthwhile to spend the time necessary to select large, extremely uniform sized test groups. By reducing dispersion around the mean and increasing group size, significant growth difference might be more likely to be found in a relatively small experimental period.

Histological Studies

Methods

Samples of tissues from the five species (Crassostrea, Hydroides, Brevoortia, Fundulus, and Callinectes) were saved for histological examination. The tissues or whole organism (Hydroides) were preserved in Davidson's fixative (Humason, 1967) for a minimum of 48 hours. After fixation, the tissues were dehydrated in graded alcohol series, cleared in xylene, and embedded in tissue mat paraffin. Tissue blocks were sectioned between 7 and 10 μ with a rotary microtome. The sections were mounted and stained using Harris' hematoxylin and eosin (Thompson, 1966). Slides were examined to detail tissue abnormalities related to chaff exposure. The results are detailed in textual form.

Results and Discussion

Crassostrea virginica

Transverse sections of test and experimental oysters showed mantle, gill, stomach, intestine, and digestive diverticula. The stomach and intestine of both control and experimental organisms showed copious amounts of food particles in the lumens. This indicates that both groups were definitely assimilating food and not shunting the algae off in pseudofeces. There is no direct cellular damage caused by the chaff. It is interesting to note that at least half the individuals showed gonadal tissue at this young age.

Callinectes sapidus

The slides of the blue crab digestive system are dominated by transverse and longitudinal transverse sections of digestive glands. The stomachs did not cut well due to the presence of chitinous gastric mills, which did not soften in fixation. Examination of the digestive glands indicated the presence of elongated hepatic cells, dark, granular basal cells, and highly vacuolated secretory or absorptive cells. Vacuolation of the hepatic cells appears to be the same for both control and experimental crabs, indicating similar physiological conditions with regard to nutrition. As with other organisms, there is no chaff present or indication of cellular damage.

Brevoortia tyrannus

The majority of the slides show the stomach characterized by prominent circular musculature and large, deep lamina propria or villi. The columnar epithelial cells have more prominent filamentous edges and a mucoid layer that is absent in Fundulus. The difference is likely due to the fishes' dietary behavior since Fundulus is an omnivore and menhaden is a filter feeder. The stomach and intestines of both control and experimental fish contained food. Again there was no evidence of direct cell damage or chaff present in any slides.

Fundulus heteroclitus

Excised tissues were cut in cross sections so that esophagus, stomach, intestine, and liver are evident on slides. Intestine is readily discernible from stomach by the larger number of goblet cells present. Parenchymal cells in the liver of some experimental animals appear to be more vacuolated than control animals. Couch (1975) reported that vacuolation is common

in fish that are stressed by various pesticides. It is most likely that in these fish the vacuolation is actually individual variation. There is no evidence of direct cellular damage by chaff on the digestive system or gills.

Hydroides dianthus

Comparison of control and experimental animals showed no outstanding differences. Tissues were oriented so that the majority of the slides were longitudinal, transverse sections of the entire worm. Because experimental organisms were collected after death, it is possible that subtle cellular differences were occluded by decomposition. However, it was possible to detect slight differences in the vacuolation of the gastric columnar epithelium. Experimental organisms were more highly vacuolated that control organisms. Ripe males and females were present in both groups. Food was present in the intestinal lumen in a majority of the slides.

Trace Metal Analysis

Initial toxicity studies conducted for SCI, Inc. have indicated that aluminum chaff suspensions can be acutely toxic and can inhibit basic physiological functions, such as feeding, for selected marine organisms. Although the direct cause of such phenomena may not be directly attributable to abnormally high concentrations of trace metals, the potential for an aluminum alloy coated product to leech relatively high concentrations of metals to ambient seawater has been adequately demonstrated in this preliminary study. Furthermore, demonstration of enhancement of several trace metals, including aluminum, in animal tissue has provided basis for hypothesizing a potential role of trace metals in both mortality and abnormal physiological phenomena among estuarine invertebrates. It is obvious that such a cursory survey as presently conducted cannot adequately be used to ascertain the role trace metals may have in the biotic response of estuarine organisms to exposures of aluminum covered chaff. Only a more detailed and in depth study designed to include replication, tissue accumulation of metals at several time intervals, a greater variety of animals, a trophic analysis of metals, including phytoplankton and other particulates, and inclusion of several additional metals could begin to describe the role of trace metals in biotic response.

Methods

In general, the experimental design for trace metal analyses in the present study interfaced with that described in the methods section for the oyster larvae and oyster feeding experiments. Since massive mortality of oyster larvae was not initially anticipated, only juvenile oysters were available for tissue analyses. As this metals' survey was initiated without funding and therefore designed as a preliminary index of trace metal

concentrations, animal tissue other than juvenile oysters was not analyzed nor were several metals extensively analyzed from the dissolved phase of seawater.

Water Sample Analysis

All water samples were filtered through 0.45μ nucleopore membrane filters, acidified and frozen immediately after collection. Sample preparations and analyses were conducted in an ultra clean laboratory facility. Samples were first chelated and dissolved with APDC and MIBK, extracted using 1% APDC solution, and pH adjusted individually for each metal ion. Extracted and concentrated samples were digested in teflon labware using concentrated, double quartz distilled nitric acid. After being brought to volume, all samples were analyzed using a carbon rod oxidation furnace (flameless) unit of a Perkin-Elmer Model 603 atomic absorption spectrophotometer having an automatic sample injection system and a computerized data entry and print-out system.

Tissue Sample Analysis

All tissue samples were freeze dried and ground with an acid-rinsed, agate-lined mortar and pestle. Subsamples of approximately 0.5 g were ashed in an oxygen-plasma system for 12 to 20 hours (until completely ashed). Ashed tissue was then transferred on a clean bench (i.e. teflon coated, laminar flow-scrubbed air) to a teflon (T.F.E.) bomb, to which 3 to 4 ml of 70% double distilled nitric acid had been added. The sealed bomb was then placed in a water bath at 90-100°C for 2 hours. The bomb was opened and rinsed with double distilled, deionized water (three rinses). The sample, on final rinse, was filtered through a 0.45 nucleopore filter and the filtrate transferred to a teflon 50 ml volumetric flask and brought to volume with double distilled water.

All reagents used for analyses were double, quartz distilled. All teflon labware was initially washed with detergent followed by five rinses in double quartz distilled water. Containers were soaked in 1:1 double distilled nitric acid followed by five to ten rinses with double quartz distilled water. Finally, all labware was washed with 1:1 hot double distilled hydrochloric acid and air dried under a clean air bench.

Results and Data Interpretation

Dissolution of Trace Metals from Chaff

Dissolution of copper, cadmium, and zinc was found at all concentrations of aluminum chaff tested. Copper concentrations in seawater increased over controls (no chaff) by factors of 25.8 and 35.4 for chaff concentrations of 100 and 1000X, respectively (Table 19). Similar increases for cadmium were 3.3 and 6.7 while zinc concentrations were 2.1 and 1.8.

Using the pulverized form of chaff resulted in an approximate two-fold increase in dissolved copper concentration (Table 19). Dissolution rates of both cadmium and zinc, however, were not affected by the change from intact to pulverized chaff.

Concentrations of dissolved metals in the culture system were found to be high relative to concentrations measured in Delaware Bay water (Table 19). Since Broadkill River water, the base for culture water used in all experiments, was not analyzed for metals, it is reasonable to assume that Broadkill water, which has homes and at least one industrial operation on its immediate shore, may have relatively high concentrations of metals. In addition, concentrations of dissolved trace metals in closed or semi-closed culture systems in general have been found to have higher metals' concentrations than contained in the original water mass (Sick, 1977).

This phenomenon is probably due to: 1) manipulation of seawater through various holding tanks, pipes, pipe fittings, pumps; increased aeration through handling, aeration from compressors (particularly contaminating for cadmium and zinc), and passage through filters, and 2) build up of inorganic and organic residues.

Trace Metal Analyses of Oyster Tissue Following Exposure to Aluminum Chaff

Enhancement of trace metals was found in all oyster tissue (total organic matter) analyzed (Table 20). Aluminum concentrations increased by approximately factors of 2 and 2.8 following extended exposure to 100 and 1000X concentrations of chaff, respectively. Likewise, copper concentrations in tissue approximately doubled at a treatment of 100X and at 1000X were 2.5 times higher than a control group. Zinc concentrations, although less than either aluminum or copper, were 28 and 73% higher than controls for oysters exposed to 100 and 1000X chaff concentrations. Although tissue concentrations of cadmium tended to be proportional to ambient chaff concentrations, no significant increases in whole oyster tissue were found. In general, tissue concentrations were higher at both 100 and 1000X concentrations of chaff among oysters exposed to pulverized chaff.

Aluminum, although not normally considered a toxic substance when present in trace amounts, has not been extensively enough studied to allow meaningful evaluation of its total short, and especially, long term effects in biological systems. Under certain circumstances, relatively low concentrations of aluminum complexes in aqueous systems can be toxic to aquatic organisms. For example, Freeman and Everhart (1971) reported that concentrations of aqueous aluminum complexes in excess of 1.5 ppm caused significant physiological abnormalities and high mortality rates

in rainbow trout fingerlings. Although concentrations of dissolved aluminum in culture water was not measured, enhancement of aluminum in oyster tissue above those found in control oysters and above concentrations found in oysters inhabiting Delaware Bay indicate probable enhancement in the dissolved phase. Furthermore, since tissue concentrations in oysters exposed to 1000X chaff had aluminum concentrations three times greater than those not exposed to chaff and 6.5 times greater than oysters from Delaware Bay, it is reasonable to hypothesize potential physiological abnormalities due to aluminum stress.

Biological effects of both copper and zinc have been well documented among marine organisms. Copper is well known to be highly toxic at relatively low concentrations. Retardation of growth and inhibition of development in ovigerous females occurred when the marine copepod, Tigriopus japonicus, was subjected to copper concentrations of 10 ppb (D'Agostino and Finney, 1974). Dissolved copper concentrations of 20, 40, and 80 ppb all proved fatal to the marine polychaete, Nereis diversicolor, after 24 hours of exposure (Jones, et al., 1976). Newly hatched Gammarus sp. (amphipod) experienced significantly high mortalities when exposed to 6 to 12 ppb of ionic copper (Arthur and Leonard, 1970). In summary, a wide variety of marine organisms have experienced severe stress resulting in mortality when exposed to copper concentrations between 10 and 100 ppb. Since concentrations directly attributable to dissolution from aluminum chaff were 60 to 160 ppb (Table 20), it is more than plausible to assume that mortality could have resulted from copper poisoning. In addition, the accumulation of copper in live oyster tissue by nearly a factor of 1000 in treatments having copper chaff suggests the strong possibility of severe physiological and biochemical abnormalities.

Although zinc is a required trace element necessary for normal physiological and biochemical function of a wide variety of enzymes at concentrations in excess of normal physiological requirements, it can be highly toxic to most marine invertebrates. Cairns and Dickson (1970), for example, have noted acute toxicity among aquatic protozoans after 1 hour's exposure to 24 ppm zinc. Most investigations have indicated that several ppm are necessary for toxicity in marine invertebrates. However, Sprague (1964) reported toxicity at 0.6 ppm zinc for the Atlantic salmon. The fact that tissue concentrations of zinc among oysters exposed to aluminum chaff are two to three times higher than those of oysters in Delaware Bay (Table 20) suggests at least sublethal abnormalities may occur from long periods of exposure in chaff. However, if the dissolution rate of zinc from chaff was rapid and the flushing rate in the environment was large, studies with several marine invertebrates suggest that elevated zinc concentrations in tissue would dissipate rapidly.

Although no accumulation of cadmium was found in oyster tissue, dissolution of cadmium from aluminum chaff (Table 19) could be accumulated by some organisms, particularly zooplankton. Sick and Baptist (1977) reported a significant accumulation of cadmium by marine copepods at seawater concentrations as low as 0.10 ppb.

Table 19

Metal analyses of water used in 48-hour oyster larval bioassay. Means and standard deviations are based on five replicate determinations per sample.

Treatment	Metal Concentration (ppb)				
	Cu	Cd	Zn	Fe	Mn
Control Tank	2.4± 0.10	1.5± 0.009	81± 3.41		
100X Aluminum Chaff (long)	62.0± 0.41	5.0± 0.04	170±11.22		
1000X Aluminum Chaff (long)	85.0± 1.11	10.0± 0.09	130± 9.82		
100X Aluminum Chaff (pulverized)	160.0±12.00	4.0± 0.31	91± 1.14		
1000X Aluminum Chaff (pulverized)	120.0± 9.12	6.0± 0.42	101± 6.42		
Sea Water, Univ. of Del. Mariculture Project	9.0± 0.48	0.28±0.011	31± 1.41	20± 1.19	.93±0.04
Del. Bay Water	0.12±0.01	0.03±0.002	0.06±0.004		

Table 20

Metal analyses of juvenile oyster tissue after exposure to aluminum chaff for 25 days. Means and standard deviations are based on five replicate determinations per sample.

Treatment	Metal Concentration (ppm)			
	Al	Cd	Cu	Zn
Control	90± 6.50	0.700±0.06	382±21.01	989± 72.70
Control	188±16.10	0.676±0.07	381±29.92	972± 89.23
100X	231±19.56	0.721±0.04	751±31.11	1250± 99.21
100X	289±21.71	0.802±0.03	851±42.60	1180± 98.23
1000X	356±19.11	0.911±0.04	1117±50.19	1622±100.21
1000X	375±29.81	0.899±0.07	952±81.61	1816±130.41
Adult Oyster Tissue from Del. Bay	55± 4.20	0.765±0.04	35± 2.11	486± 34.62

Conclusions

Projection of Results of Long Term or Continued Exposure

Since the experiments were designed to measure the immediate, short term effects of chaff on typical estuarine species common to the Navy Research Laboratory Radar Testing Site, Chesapeake Beach, Maryland, projection of results to long term or continued exposure are based on these short term experiments, past bioassay experience, and knowledge of the literature. As a result, these projections must be necessarily restrictive and are presented in a cautious mode. In addition to the biotic response of the organisms to long term exposure, we know so little about the chemistry of chaff in seawater and the frequency and volume of chaff placed in the environment, that we have almost no basis to offer projections of long term exposures.

One could recommend a series of long term experiments at high concentrations for the present species which would subsequently reveal their LD_{50} levels. Such information would be valuable, but if this were the only approach used, one would find that any organism can be stressed this way. What we have done is to indicate the relative vulnerability of the species to the short term experiments and which aspects of their biology might be more revealing to testing under long term exposure conditions. We consider this a more viable approach then merely suggesting longer time and higher concentrations across the board.

Crassostrea virginica

Since mortality was 100% in all experiments with 0-48 hour larvae, discussion of long term exposure is irrelevant with this stage. Ten day old larvae were hardier than 48 hour larvae. However, there is evidence to suggest that the growth of 10 day old larvae might have been adversely affected by the short term

experiments. We suspect that this effect would be accentuated in a long term experiment. In regard to the ingestion of algae by juvenile oysters the majority of evidence indicated that there was no difference between controls and test organisms. However on 3 of 16 experiments test organisms ingested less algae than controls. These feeding experiments may deserve refining and duplication. Pending further chemical analysis of the disassociation of chaff in seawater, a schedule of sublethal experiments might be warranted.

Mytilus edulis and _Hydroides dianthus_

There was strong evidence to indicate that even short term exposures of chaff under present test conditions were lethal to the polychaete, _H. dianthus_. At these concentrations long term exposure of _H. dianthus_ to chaff would probably be unnecessary to bracket lethal doses. There was some evidence to suggest that if present concentrations of chaff were not lethal to an individual polychaete that its behavior was affected. We would recommend sublethal studies here also.

In regard to _M. edulis_, mortalities under these concentrations were so low that chaff did not adversely affect them. Larvae and juveniles might be another matter. Regardless, adults of _M. edulis_ are efficient filter feeders and they are known to accumulate high concentrations of trace metals without extensive mortality. This species is presently being used by EPA in its worldwide mussel watch as a bioassay organism for trace metals. Once again we submit that experiments designed to test sublethal effects would be much more meaningful. For example, feeding rates and byssal attachment are important biological processes which could be tested rather easily. Any adverse tampering with feeding and byssal attachment would ultimately lead to lower survival rates.

Callinectes sapidus

It would have been preferable to conduct the same experiments prior to the winter hibernation of the crabs. Regardless, there was no evidence to indicate that the mortality of adult blue crabs was attributable to chaff under present test conditions. In regard to feeding there was some indication of reduced food ingestion at the highest concentration. We believe that long term feeding experiments would affect feeding and other behavior. It should be mentioned that crustacean larvae are extremely sensitive to pollutants. Although adult crabs were apparently unaffected by the present test conditions, we submit that larval stages of blue crabs might be extremely vulnerable to chaff. As such, long term exposures would be even more injurious.

Brevoortia tyrannus and *Fundulus heteroclitus*

Based on present test conditions the relationship between mortality of menhaden and chaff was inconclusive. Mortalities among killifish were apparently unrelated to test conditions. Present experiments were definitely too short to demonstrate significant change in total length and live weight for control and test organisms. Prior to any projections of long term exposures brackets for short term effects would have to be precisely defined.

Histological Studies

Based on histological sections there was no evidence of damage to the oyster, blue crab, menhaden, and killifish by chaff under these test conditions. In contrast, there were some slight differences in the gastric columnar epithelium between control and test organisms of *H. dianthus*. We would project that long term exposure would adversely affect the fish, crab, and oyster. Since present test conditions were seriously stressing *H. dianthus*, long term exposure would only accentuate these effects. Long term exposures at lower concentrations would be more valuable with this species.

Projection of the Probability of Environmental Impact on the Species Tested or the Natural Food Chain

A caveat similar to the one expressed for projection of long term exposures is also presented here. Since no laboratory or field food chain studies were conducted and trace metal analyses were only run on juvenile oysters, only some broad generalizations can be made based on short term experiments. The movement of copper, zinc, chromium, lead, and mercury has been studied by others in laboratory food chains involving phytoplankton, zooplankton, mussels, and mice. Toxicity was induced in the organisms at the upper ends of the food chain through transmitted movement of the pollutant. Some marine organisms are able to purge themselves of trace metals when the source of trace metals is removed.

In regard to the test species, the mussel and the oyster are efficient filter feeders and are capable of accumulating high concentrations of trace metals from the water column, phytoplankton, and particulate matter stirred up from the bottom. The other filter feeder, *H. dianthus*, is probably capable of accumulating trace metals, but little is known about its actual response compared to the better studied oyster and mussel. Since the blue crab and killifish are omnivores they would be expected to accumulate trace metals primarily through other organisms, although there is some evidence to suggest that the blue crab could accumulate some trace metals directly from the water column. The menhaden poses an interesting feeding type in that it is a filter feeder which seems to prefer zooplankton as a juvenile changing its habit to phytoplankton as an adult. As such, it has several pathways for the accumulation of trace metals.

Exclusive of the blue crab and killifish, there is probably very little trophic interaction among the test species. Blue crabs can feed on all the

test organisms in juvenile or adult stages and are known predators of oysters and other bivalves. The killifish would be capable of scavenging the other test organisms. Juvenile menhaden might inadvertently feed on oyster larvae, but the degree of this ingestion is unknown.

In natural food chains the probability of trophic interaction would be considerably greater. There are many typical estuarine fish and crabs known to feed upon oysters, mussels, and polychaetes. Some common larger species of fish feed on smaller species of fish and crabs. In turn, marine mammals and other top carnivores feed on the large fish. When the marine mammals and large fish perish, they are scavenged by crabs and smaller species of fish originally included in the food chain. Many of these loops occur in marine food chains so that pathways are so complex that they are almost impossible to trace in real world situations.

In principle, we submit that chaff and its trace metals could be incorporated in estuarine food chains. However, we know so little about its chemistry, volume, and frequency of placement that we cannot project whether accumulation in food chains will be harmless or deleterious to that particular ecosystem. We suspect that natural sources of trace metals and other human activities exceed the effect of chaff on the Chesapeake estuarine system.

Summary

1. Aluminum fiberglass covered chaff is lethally toxic to 0-48 hour old oyster larvae and the filter feeding polychaete, Hydroides dianthus.

2. Several sublethal effects have been documented. Feeding rates in the oyster, Crassostrea virginica were depressed by the presence of chaff in three of sixteen experiments. The menhaden, a filter feeding fish, lost weight in 1000X concentrations of chaff.

3. Although the evidence is not conclusive, it appears that by lengthening the experimental period, growth and weight differences would be significant for most species tested.

4. Histological examination of tissues showed no chaff present or indication of direct cellular damage by chaff. More subtle cellular response to stress was not noted with the exception of highly vacuolated parenchymal and hepatic cells in Fundulus and Callinectes respectively.

5. The larger omnivorous species such as Callinectes and Fundulus appear to be least affected by the presence of chaff.

6. It appears that leeching of toxic trace metals from the chaff is primarily responsible for the biological effects noted, while mechanical or abrasive damage is of a secondary nature.

7. The data presented in this study has shown a need for further experimentation regarding the biotic response to chaff, particularly of filter feeders.

8. In design of new experimental programs emphasis should be placed on: a) development of flow-through test systems, b) trace metal analysis of chaff during its aging process in seawater, c) trace metal uptake by test organisms, d) determination of a standard volumetric, rather than area concentration factor, e) species selection to include plankton and larval stages which may be highly susceptible to chaff.

Literature Cited

Arthur, J.W., and E.N. Leonard, 1970. Effects of copper on Gammarus pseudolimnaeus, Physa integra, and Campeloma decisum in soft water. Fish. Res. Bd. Canada 27(7): 1277-1283.

Becker, C.D., and T.O. Thatcher, 1973. Toxicity of power plant chemicals to aquatic life. U.S. Atomic Energy Commission, Publ. 1249. 218 pp.

Bigelow, H.B., and W.C. Schroeder, 1953. Fishes of the Gulf of Maine. Fishery Bulletin 74. Fish. Bull. U.S. Fish & Wildl. Serv. Vol. 53.

Cairns, J., and A. Scheier, 1958. Effects of temperature and hardness of water upon toxicity of zinc in the snail Physa heterostrophia. Motulae maturae 308: 1-11.

Castagna, M., and P. Chanley, 1973. Salinity tolerance of some marine bivalves from inshore and estuarine environments in Virginia waters on the western mid-Atlantic coast. Malacologia 12(1): 47-96.

Costlow, J.D., and C.G. Bookout, 1959. The larval development of Callinectes sapidus Rathbun reared in the laboratory. Biol. Bull. 116(3): 373-396.

Couch, J.A., 1975. Histopathological effects of pesticides and related chemicals on the livers of fishes. In: The Pathology of Fishes, Ed. W.E. Ribelin and G. Migaki, Wisconsin Press. 559-584 pp.

Danforth, H., 1976. Future of shellfish culture in Washington. Sea Grant 70's 7(1).

Day, S.H., 1973. New Polychaeta from Beaufort with a key to all species recorded from North Carolina. NOAA Tech. Rept. NMFS Circ-375.

Epifanio, C.E., and R.F. Srna, 1975. Toxicity of ammonia, nitrite ion, nitrate ion, and orthophosphate to Mercenaria mercenaria and Crassostrea virginica. Mar. Biol. 33: 241-246.

Freeman, R.A., and W.H. Everhart, 1971. Toxicity of aluminum hydroxide complexes in neutral media to rainbow trout. Trans. Am. Fish. Soc. 100(4): 644-658.

Gonzalez, J.G., and P. Yevich, 1976. Responses of an estuarine population of the blue mussel, Mytilus edulis, to heated water from a steam generating plant. Mar. Biol. 34: 177-189.

Humason, G.L., 1967. Animal Tissue Techniques. W.H. Freeman and Company, San Francisco, 569 pp.

Jones, L.H., M.V. Jones, and A.J. Radlett, 1976. Some effects of salinity on the toxicity of copper to the polychaete, Nereis diversicolor. Estuarine and Coastal Marine Science 4: 107-111.

Keck, R.T., R.C. Heess, J. Wehmiller, and D. Maurer, 1977. Sublethal effects of Nigerian crude oil on the juvenile hard clams, Mercenaria mercenaria (Linne). Environmental Pollution (in press).

Lee, R.F., R. Saurerheber, and A.A. Benson, 1972. Petroleum hydrocarbons: uptake and discharge by the marine mussel Mytilus edulis. Science 177: 344.

Loosanoff, V.L., and H.C. Davis, 1963. Rearing of bivalve mollusks. In: Advances in Marine Biology. Ed. F.S. Russell, Academic Press, London 1: 1-136.

Loosanoff, V., and F.D. Tommers, 1948. Effect of suspended silt and other substances on rate of feeding of oysters. Science 107: 69-70.

Maurer, D., J.C. Tinsman, W. Leathem, and P. Kinner, 1976. Baseline studies of Delaware ocean outfall sites. Mar. Poll. Bull. 7(2): 31-34.

National Marine Fisheries Service, 1977. Draft Report: A comprehensive review of the commercial oyster industries in the United States. Office of Fisheries Development, Washington, D.C.

Rice, T.R., and J.R. Smith, 1958. Filtering rates of the hard clam (Venus mercenaria) determined with radioactive plankton. Fish. Bull. U.S. 58: 73-82.

Sick, L.V., 1977. Concentrations of selected trace metals in the abiotic and biotic components of a semi-closed culture system for oysters. University of Delaware, Technical Report Series, DEL-SG-5-77.

Sick, L.V., and G.J. Baptist, 1977. Accumulation of environmental concentrations of cadmium from food and water by the marine copepod Pseudodiaptomus coronatus. Limnol. and Oceanog. (in press).

Tagatz, M.E., 1969. Some relations of temperature. Acclimation and salinity to thermal tolerance of the blue crab, Callinectes sapidus. Trans. Amer. Fish. Soc. 98(4): 713-716.

Tarzwell, C.M., 1969. Standard methods for determination of relative toxicity of oil dispersants and mixtures of dispersants and various oils to aquatic organisms. In: Proceedings of Joint Conference on Prevention and Control of Oil Spills. API, FWPCA, pp. 179-186.

Thompson, S.W., 1966. Selected Histochemical and Histopathological Methods. Charles C. Thomas, Springfield, Ill.

Williams, A.B., 1965. Marine decapod crustaceans of the Carolinas. Fish. Bull. 65(1): 1-298.

Woelke, C.W., 1972. Development of a receiving water quality bioassay criterion based on 48-hour Pacific oyster (Crassostrea gigas) embryo. Washington Dept. of Fisheries, Technical Rept. 9, 1972.

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APPENDIX 2

EFFECT OF CHAFF ON THE AMERICAN OYSTER, *CRASSOSTREA VIRGINICA* AND THE POLYCHAETE WORM, *NEREIS SUCCINEA*

Final Report Submitted To:

Systems Consultants, Inc.
1054 31st Street, N.W.
Washington, D.C. 20007

Principal Investigators:

William C. Graves

Hayes T. Pfitzenmeyer

John C. Rhoderick

Willem H. Roosenburg

Philip W. Heuman, Jr.

Ronald M. Block

Center for Environmental and Estuarine Studies
Chesapeake Biological Laboratory
Solomons, Maryland 20688

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SECTION I: EXPERIMENTS WITH CRASSOSTREA VIRGINICA

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INTRODUCTION

This report describes the experimental effects of chaff upon two commonly found species in the Chesapeake Bay.

The study is being conducted for the Naval Research Laboratory under Contract N00173-76-C-307 by Systems Consultants, Inc., with the University of Maryland CEES-CBL serving as the subcontractor (proposal No. UMCEES-77-86CBL)

Chaff is the collective term for aggregates of metallic or metal coated strips or cylinders which are dispersed in the air to serve as reflectors of radio-frequency electromagnetic radiation. This material eventually reaches the earth and consequently falls into the Chesapeake Bay.

The purpose of this study was to determine the effects of one form of chaff, aluminized and non-aluminized filaments of fiber glass upon 0-48 hour oyster larvae of Crassostrea virginica and the polychaete annelid worm Nereis succinea. These species were chosen since they are commonly found at the release sites. For the purpose of this study the mobility effects of chaff were ignored in order to obtain a worst-case situation. Therefore, testing was conducted in a static water system.

SECTION I: Crassostrea virginica

GENERAL METHODOLOGY

Cultch-less Crassostrea virginica breeding stock was obtained from a hatchery. The brood stocks were acclimated for at least one week in cooled (18°C) flowing Chesapeake Bay water of 13°/₀₀ salinity. Chesapeake Bay water was also used for spawning.

Oysters were induced to spawn using temperature manipulation (increasing the temperature above ambient up to 30°C). The spawning methodology of Loosanoff and Davis (1963) was used exc. t that a flowing seawater system was used instead of a static system. Upon spawning, gametes from adult male and female oysters were collected separately.

The addition of sperm to eggs was considered time zero. Microscopic examination of five 1ml samples of the sperm and egg mixture was performed to determine quality of fertilization and density of fertilized eggs. Based on these observations, dilutions were made to obtain a suspension of approximately 300 fertilized eggs per 1ml. This was considered the base number with which each test chamber was innoculated.

Two kinds of chaff were used in both studies: aluminized and non-aluminized. All chaff material used was 16mm in length. Test concentrations of chaff were based on the maximum predicted amount (53 dipoles per square foot of water surface) which occurred in actual field deployment of the material. Based on this and the water surface area of the beakers (.102 square feet) experimental concentrations for aluminized chaff of 1x, 10x and 100x required 0.0006g (5.406 dipoles), 0.006g (54.06 dipoles) and 0.06g (540.6 dipoles), respectively. Due to differences in make up between aluminized (coated fiber) and non-aluminized chaff (uncoated fiber) weights of only 0.0003g, 0.003g and 0.03g were required for the same test concentrations using the non-aluminized chaff.

The experimental chambers consisted of 1-liter glass beakers which were precleaned with a 200 ppm sodium hypochlorite solution, rinsed in tap water and air dried.

The experiment began when the appropriate concentrations of chaff were added to 900ml of 1μm filtered test water 30 minutes prior to the injection of the one hour old oyster larvae.

Upon termination of each 48 hour bioassay water from each control and test chamber was poured separately through a 30 μm Nytex sieve to catch

and concentrate the larvae. The larvae were decanted into 15 ml vials and preserved with 1% formalin. The methodology of Calabrese et al., (1973) was used to determine survival except that the entire collected sample was counted in a Sedgwick-Rafter cell instead of . ml representative aliquots. Also an alternative method (based on live-count surviving)for determining survival is presented in Appendix A.

The following water quality parameters were monitored at the time zero and at 24 hour intervals in accordance with the procedures outlined in Standard Methods 1976: temperature (continuous recording), dissolved oxygen, pH, salinity and ammonia-nitrogen.

EXPERIMENTATION

Experiment I: Range Find

This first experiment was performed as a preliminary study to first, establish an efficient methodology; second, find a suitable test media and third, to establish at what concentration of chaff toxic effects are observed.

Two series of 48 hour bioassays investigating 10x and 100x concentrations of chaff were conducted concurrently and in triplicate: One used Chesapeake Bay water, the other synthetic seawater diluted to 13.2°/oo salinity (Standard Methods, 1976). The oysters that provided the gametes for experimentation in synthetic seawater were rinsed in synthetic seawater during the transfer from spawning tank to the gamete collection aquaria. Male and female gametes were collected in the appropriate test water. Sperm and eggs were combined and the number of fertilized eggs determined by taking the average of five 1 ml counts. In the synthetic seawater-chaff experiment dilutions were performed so that 258 eggs per beaker were injected, whereas in the Chesapeake Bay water-chaff experiment an inoculum of 316 was used. (See Appendix B).

Experiment II.

The results from the first experiment tentatively indicated to us that the 10x and 100x concentrations of chaff exhibited some type of toxic effects on 0-48 hour oyster larvae (See Table 1). Results also showed Chesapeake Bay water superior to synthetic seawater as the test medium - Mean percent of oyster larvae recovered was 40.1 in Chesapeake Bay water while only 20.0 in synthetic seawater. Consequently, synthetic seawater was discontinued as a test medium.

Therefore, to investigate whether toxicity was a result of a direct contact with the chaff or a result of a toxic substance from the chaff dissolving into the test water, a second bioassay was performed. Based on the preliminary results obtained from Experiment I, two test concentrations of chaff were used: 1x and 10x.

Bioassay procedures were the same as in Experiment I with the following modifications: Spawnable *Crassostrea virginica* were not available in the Chesapeake Bay area due to the early culmination of the spawning season. Seasonal water temperature at the time of the second experiment had increased above the natural spawning temperature. Two attempts were made with local natural populations without success. Therefore, to obtain spawnable broodstock of the same test species; *Crassostrea virginica* were obtained from the NMFS hatchery, Milford, Connecticut. Three hundred fertilized eggs were placed directly into control and test beakers (uncaged) or into cages (glass cylinders with a 30 μm Nytex screen on the bottom) suspended within the control and test beakers. (see Appendix B). Uncaged studies allowed direct contact of larvae to chaff while suspended caged studies allowed indirect contact by letting water exposed to the chaff to flow freely into the cages containing oyster larvae. Control and experimental tests were conducted in quadruplicate to allow for good statistical interpretation.

RESULTS AND DISCUSSION

Experiment I.

Table 1 contains larval survival data for the first 0-48 hour Range Find bioassay. As shown in this table both aluminized (coated) and non-aluminized (uncoated) chaff exhibited toxic effects on oyster larvae at both the 10x and 100x concentrations. In addition, Chesapeake Bay water was found to be superior to synthetic seawater as a test medium. However, since this was only a preliminary investigation to determine the range of concentration of chaff toxic to oyster larvae as well as to establish which test media was best; no statistical interpretation of the results was made. Results from this experiment will therefore be disregarded in trying to ascertain the toxic effects of chaff. The use of the third test beaker in each group for determining water quality parameters (where contamination could occur) diminished the sample size to two, further justifying the lack of statistical interpretation.

Ranges of water quality taken during the 48 hour bioassay are given in Table 2. Temperature ranged between 26.5°C; salinity increased 2% or less; dissolved oxygen decreased a maximum of .6 ppm which is a rate likely to occur in a static test system; pH fluctuated only .2 which is well within the tolerance limits for *Crassostrea virginica* oyster larvae. Ammonia-nitrogen levels which varied from one test chamber to another were also well within tolerance levels. No differences in water quality among control, aluminized (coated) and non-aluminized (uncoated) test beakers were observed.

EXPERIMENT II.

Data for the bioassay investigating the mode of toxicity of chaff to 0-48 hour oyster larvae appear in Table 3. Statistical comparisons using the One Factor Analysis of Variance test are given in Table 4.

If the value of F (given in table) exceeds 3.78 we reject the hypothesis of equal means at the 90% confidence level. If, however, the value of F exceeds 5.99 we reject the hypothesis of equal means at the 95% confidence level (Degrees of Freedom in both instances = 1,6).

Results indicate that there is a significant difference (95% confidence level) between the uncaged control and the uncaged 10x non-aluminized (uncoated) samples; the caged control and the caged 1x aluminized (coated) and the caged 10x aluminized (coated)samples. At the 90% confidence level statistical significance is found between: The uncaged control and the uncaged 1x aluminized (coated) samples; the uncaged controls and the uncaged 10x aluminized (coated) samples; the uncaged 1x non-aluminized (uncoated) and the uncaged 10x non-aluminized (uncoated) samples; the caged 1x non-aluminized (uncoated) and the caged 1x aluminized (coated) samples; and the caged control and uncaged control samples.

Therefore, results from this experiment can be summarized as follows:

1) 10x Non-aluminized chaff, 1x Aluminized chaff and 10x Aluminized chaff exhibit a toxic effect when in direct contact with 0-48 hour oyster larvae of <u>Crassostrea virginica</u>.
    a) 1x Non-aluminized chaff when in direct contact with larvae exhibit no toxic effects.
2) The toxic effect exhibited in the 1x Aluminized chaff caged experiment is not conclusive because no effect was found at the 10x concentration.
3) Therefore, both non-aluminized and aluminized chaff when not directly in contact with the oyster larvae show no significant effects.
4) There is a difference between caged and uncaged controls which may indicate that the cages themselves may exert some factor on larval survival.

Ranges of water quality taken during this 48 hour bioassay are given in Table 5. These measurements were taken from an uncaged and caged water quality control beaker. As the results from Experiment I indicate, taking water quality measurements may have a possible deliterious effect on oyster

larval survival. Counts made from these additional beakers were not included in the results. Therefore, the sample size used in statistical interpretation was four in both control and test groups.

Temperature remained constant at 26°C; salinity increased 2%; dissolved oxygen decreased a maximum of .2 ppm; pH increased .3, which is well within the tolerance limits for <u>Crassostrea virginica</u> oyster larvae; Ammonia-nitrogen levels, which varied from one test chamber to another, were also well within tolerance levels. No significant differences in water quality were found between Experiment I and Experiment II.

TABLE 1

EXPERIMENT I: Results of the 48 hour exposure to 10x and 100x chaff on 0-48 hour larvae of *Crassostrea virginica*. Mortality is expressed as a percentage of the average number surviving in the control cultures.

Synthetic Seawater

	Sample #	#Live	#Dead	% Total Recovered	$\bar{x}$ Live *	Mortality
Control	1	95	13	41.9	47.7	N/A
	2	48	1	18.1		
	3	0	0	0		
Non-Aluminized Chaff (10x)	1	0	4	1.6	37.3	.218
	2	98	27	48.4		
	3	14	6	7.7		
Non-Aluminized Chaff(100x)	1	4	16	7.7	1.3	.973
	2	0	2	0.8		
	3	0	4	1.6		
Aluminized Chaff(10x)	1	0	0	0.0	.67	.986
	2	2	5	2.7		
	3	0	1	0.38		
Aluminized Chaff (100x)	1	0	0	0.0	.67	.986
	2	2	2	1.6		
	3	0	0	0.0		

* $\bar{x} = 1/3 \sum^{3}$ (No. live in Replicate i) $= 1/3 \sum_{i=1}^{3} x_i$

TABLE 1 (continued)

Chesapeake Bay Water

	Sample #	#Live	#Dead	% Total Recovered	$\bar{x}$ Live *	Mortality
Control	1	253	16	85.1		
	2	90	11	31.9	134.3	N/A
	3	60	7	21.2		
Non-Aluminized Chaff (10x)	1	82	4	27.2		
	2	73	7	25.3	68.7	.488
	3	51	9	18.9		
Non-Aluminized Chaff (100x)	1	42	7	15.5		
	2	84	5	28.1	65.9	.514
	3	70	8	24.7		
Aluminized Chaff(10x)	1	17	10	8.5		
	2	40	18	18.4	20.3	.849
	3	4	5	2.8		
Aluminized Chaff(100x)	1	0	3	0.9		
	2	2	4	1.9	.67	.995
	3	0	2	0.6		

TABLE 2

Synthetic Seawater

EXPERIMENT I: Ranges of water quality (data taken from the third test beaker)

	Temp °C	Salinity °/₀₀	D.O. (ppm)	pH	NH_3-N (ug)
Control	26.5-26.0	12.0 - 12.2	7.3 - 6.8	7.9 - 7.6	.11 - .05
Non-Aluminized Chaff(10x)	"	12.7 - 12.9	7.3 - 6.7	8.0 - 7.7	.09 - .04
Non-Aluminized Chaff(100x)	"	15.0 - 15.1	7.2 - 6.7	8.0 - 7.8	.08 - .04
Aluminized Chaff (10x)	"	14.9 - 15.0	7.2 - 6.8	8.0 - 7.8	.1 - .03
Aluminized Chaff (100x)	"	14.9 - 15.1	7.1 - 6.7	8.0 - 7.8	.05 - .01

Chesapeake Bay Water

	Temp °C	Salinity°/₀₀	D.O. (ppm)	pH	NH_3-N (ug)
Control	26.5-26.0	13.3 - 13.4	7.2 - 7.0	7.9 - 8.0	.1 - .008
Non-Aluminized Chaff (10x)	"	13.3 - 13.5	7.2 - 6.9	7.9	.1 - .01
Non-Aluminized Chaff(100x)	"	13.6 - 13.6	7.2 - 6.8	7.9	.05 - .02
Aluminized Chaff (10x)	"	13.2 - 13.5	7.2 - 6.8	7.8 - 7.9	.16 - .02
Aluminized Chaff (100x)	"	13.2 - 13.5	7.2 - 6.8	7.7 - 7.9	.13 - .02

TABLE 3

EXPERIMENT II: Results of the 48 hour exposure to 10x and 100x chaff on 0-48 hour larvae of *Crassostrea virginica*. Mortality is expressed as a percentage of the average number surviving in the control cultures. (Calabrese et al., 1973)

UNCAGED

	Sample #	#Live	#Dead	% Live Recovered	$\bar{x}$ Recovered Live*	Mortality
Control	1	197	80	67.7	146.5	N/A
	2	126	67	64.3		
	3	104	69	57.6		
	4	159	53	70.6		
Non-aluminized (1x)	1	111	42	51.0	121.75	16.9
	2	113	63	58.6		
	3	81	39	40.0		
	4	182	55	79.0		
Non-aluminized(10x)	1	64	32	32.0	75.25	48.6
	2	90	26	38.6		
	3	94	41	45.0		
	4	53	10	21.0		
Aluminized (1x)	1	100	41	47.0	94.25	35.7
	2	68	33	33.6		
	3	114	33	49.0		
	4	95	43	46.0		
Aluminized (10x)	1	91	11	34.0	95.0	035.2
	2	96	32	42.6		
	3	72	39	37.0		
	4	121	40	53.6		

$* = \bar{x} = 1/3 \sum^{3}$ (No live in Replicate i) $= 1/3 \sum_{i=1}^{3} x_i$

TABLE 3 (continued)

CAGED

	Sample #	#Live	#Dead	% Live Recovered	$\bar{x}$Recovered Live*	Mortality
Control	1	99	49	49.3	99.25	N/A
	2	116	44	53.3		
	3	98	53	50.3		
	4	84	43	42.3		
Non-Aluminized (1x)	1	159	40	66.3	111.25	N/A
	2	101	23	41.3		
	3	94	39	44.3		
	4	91	49	46.6		
Non-aluminized (10x)	1	74	20	31.3	92.5	Q6.8
	2	98	53	50.3		
	3	43	18	20.3		
	4	155	47	67.3		
Aluminized (1x)	1	73	28	33.6	77.25	22.2
	2	71	30	33.6		
	3	75	29	34.6		
	4	90	21	37.0		
Aluminized (10x)	1	120	37	52.3	109	N/A
	2	106	42	49.3		
	3	86	36	40.6		
	4	124	43	55.6		

* $\bar{x} = 1/3\ \Sigma$(No live in Replicate $i = \sum_{i=1}^{3} x\ i$

TABLE 4

Statistic comparisons using the one factor analysis of variance test. Calculations were based on the Calabrese (1973) method of determining mortality.

	F
Control uncaged vs. 1x non-aluminized, uncaged	0.706
vs. 10x non-aluminized, uncaged	9.948*
vs. 1x aluminized, uncaged	5.419**
vs. 10x aluminized, uncaged	5.492**
Control caged vs. 1x non-aluminized, caged	0.479
vs. 10x non-aluminized, caged	0.075
vs. 1x aluminized, caged	7.853*
vs. 10x aluminized, caged	0.816
1x aluminized, uncaged vs. 1x non-aluminized,uncaged	1.376
10x aluminized, uncaged vs. 10x non-aluminized,uncaged	1.940
1x non-aluminized, uncaged vs. 10x non-aluminized, uncaged	3.888**
1x aluminized, uncaged vs. 10x aluminized, uncaged	0.003
1x aluminized, caged vs. 1x non-aluminized, caged	4.181 **
10x aluminized, caged vs. 10x non-aluminized,caged	0.429
1x non-aluminized, caged vs. 10x non-aluminized, caged	0.430
1x aluminized, caged vs. 10x aluminized, caged	10.910 *
Control uncaged vs. control caged	4.918**

*Rejects the hypothesis of equal means at the 95% Confidence Level or less.

**Rejects the hypothesis of equal means at the 90% Confidence Level or less.

TABLE 5

EXPERIMENT II: Ranges of water quality (data was taken from a caged and uncaged water quality control beaker).

	Temp(°C)	Salinity (°/oo)	D.O.(ppm)	pH	NH_3-N (ug)
Caged	26.0	15.0 - 15.3	7.2 - 7.0	8.0 - 8.3	.1 - .13
Uncaged	26.0	14.9 - 15.1	7.8 - 7.6	8.0 - 8.3	.09 - .14

APPENDIX A

The Live-Count Surviving Method for Determining Mortality.

INTRODUCTION

In this section larvae survival data from Experiment II is presented using an alternative method than that of Calabrese et al., (1973). This method, unlike Calabrese's (1973), uses both the live and the dead counts of the percent remaining for determining the mean live recovered as well as the percent mortality. Recovered counts are used because the quantity of oyster larvae injected may vary depending on the quality of the stirring of innoculum (See Appendix B - Experiment II) as well as loss due to rupture caused by injecting.

CALCULATIONS

$\bar{x}$: Calculation of the mean is made as follows:

$$\bar{x} = 1/3\,\Sigma\,(\text{Fraction Line}) = 1/3 \sum_{i=1}^{3} \frac{(\text{No. live in Replicate } i)}{(\text{Total Count in Replicate } i)}$$

$$1/3 \sum^{3} \bar{x}\, i \qquad i = 1$$

MORTALITY

Mortality is then expressed as a percentage of the average number surviving in control cultures. Results based on the above methods of calculation appear in Appendix A - Table 1.

STATISTICAL ANALYSIS

The One Factor Analysis of Variance Test is then used to determine any significant statistical differences. F-values obtained by using this test are given in Appendix A - Table 2.

If the F-value exceeds 3.78 we reject the hypothesis of equal means at the 90% confidence level. If, however, the value of F exceeds 5.99 we reject the hypothesis of equal means at the 95% confidence level. Degrees of Freedom = 1,6 in both instances.

RESULTS

Based on Appendix A-Table 2, results can be summarized as follows:

1) There is no significant difference between caged and uncaged controls.

2) At the 95% confidence level we reject the hypothesis of equal means between the control and 10x aluminized and 10x non-aluminized samples for both the caged and uncaged test conditions.

3) At the 90% confidence level we reject the hypothesis of equal means between the caged control and the caged 1x aluminized chaff sample.

4) At the 95% confidence level we reject the hypothesis of equal means between the 1x and 10x concentrations for both aluminized and non-aluminized chaff in caged and uncaged test conditions.

5) There is no significant difference between aluminized and non-aluminized at both 1x and 10x concentrations in the caged and uncaged test samples.

TABLE 1

EXPERIMENT II: Results of the 48 hour exposure to 1x and 10x chaff on 0-48 hour larvae of *Crassostrea virginica*

UNCAGED

	Sample #	#Live	#Dead	Total	$\bar{x}$ Recovered Live*	Mortality	
Control	1	197	80	277	.679	N/A	
	2	126	67	193			
	3	104	69	173			
	4	159	53	212			
Non-aluminized (1x)	1	111	42	153	.702	N/A	
	2	113	63	176			
	3	81	39	120			
	4	182	55	237			
Non-aluminized (10x)	1	64	32	96	.254	37.4	
	2	90	26	116			
	3	94	41	135			
	4	53	10	63			
Aluminized (1x)	1	100	41	141	.711	N/A	
	2	68	33	101			
	3	114	33	147			
	4	95	43	138			
Aluminized (10x)	1	91	11	102	.239	35.2	
	2	96	32	128			
	3	72	39	111			
	4	121	40	161			

* $\bar{x} = 1/3\ \Sigma(\text{Fraction Line}) = 1/3 \sum^{3} \left(\frac{\text{No. Live in Replicate } i}{\text{Total Count in Replicate } i}\right) = 1/3 \sum_{i=1}^{3} x_i$

TABLE 1 (continued)

CAGED

	Sample #	#Live	#Dead	Total	$\bar{x}$ Live Recovered*	Mortality
Control	1	99	49	148	.676	N/A
	2	116	44	160		
	3	98	53	151		
	4	84	43	127		
Non-Aluminized(1x)	1	159	40	199	.742	N/A
	2	101	23	124		
	3	94	39	133		
	4	91	49	140		
Non-Aluminized (10x)	1	74	20	94	.272	40.2
	2	98	53	151		
	3	43	18	61		
	4	155	47	202		
Aluminized (1x)	1	73	28	101	.739	N/A
	2	71	30	101		
	3	75	29	104		
	4	90	21	111		
Aluminized (10x)	1	120	37	157	.268	39.6
	2	106	42	148		
	3	86	36	122		
	4	124	43	167		

* $\bar{x} = 1/3\ \Sigma\ (\text{Fraction Line}) = 1/3 \sum_{i=1}^{3} \frac{(\text{No. Live in Replicate } i)}{(\text{Total Count in Replicate } i)} = 1/3 \sum_{i=1}^{3} x_i$

TABLE 2

Statistical comparisons using the one factor analysis of variance test. Calculations were based on the live count method of determining mortality.

		F
Control uncaged	vs. 1x non-aluminized, uncaged	.307
	vs. 10x non-aluminized,uncaged	68.389*
	vs. 1x aluminized, uncaged	.671
	vs. 10x aluminized,uncaged	53.973*
Control caged	vs. 1x non-aluminized, caged	2.450
	vs. 10x non-aluminized, caged	128.270*
	vs 1x aluminized, caged	4.603**
	vs. 10x aluminized, caged	358.312*
1x Aluminized, uncaged vs. 1x non-aluminized,uncaged		.060
10x aluminized, uncaged vs. 10x non-aluminized,uncaged		.059
1x non-aluminized, uncaged vs. 10x non-aluminized, uncaged		86.108*
1x aluminized, uncaged vs. 10x aluminized, uncaged		73.842*
1x non-aluminized, caged vs. 10x non-aluminized,caged		88.583*
1x aluminized,caged vs. 10x aluminized, caged		288.532*
1x non-aluminized, caged vs. 1 aluminized, caged		.004
10x non-aluminized,caged vs nox aluminized,caged		.019
Control, caged vs. control, uncaged		,007

*Rejects the hypoteesis of equal means at the 95% Confidence Level or less.

**Rejects the hypothesis of equal means at the 90% Confidence Level or less.

COMMENTS AND DISCUSSION

Comparison of data between the Calabrese et al. (1973) method and the live-count surviving method is given in Appendix A - Table 3.

Results (uncaged studies) obtained by using the Calabrese et al.(1973) methodology of calculation show significant differences (95% Confidence Level) between the controls and the 10x non-aluminized (uncoated) samples. On the other hand, results obtained by using the live-count surviving method of calculation show significant differences (95% Confidence Level) between the controls and the 10x aluminized (coated) as well as between the controls and the 10x non-aluminized (uncoated) test samples.

Data for the caged studies when compared by using the two methods of calculation show marked differences in results. By using the Calabrese et al. (1973) method no significant differences are found between controls and the aluminized (coated) and non-aluminized (uncoated) test samples at both the 1x and 10x test concentrations. The live-count surviving calculation method, on the other hand, reveals significant differences at the 95% Confidence Level between the controls and the 10x aluminized (coated) samples as well as between the controls and the 10x non-aluminized (uncoated) samples.

TABLE 3

Comparison of percent mortality and statistical differences between results obtained by using the method of Calabrese et al.(1973) and the live-count surviving method.

MORTALITY

	Calabrese	Live-Count Surviving Method
Uncaged		
1x Aluminized	35.7**	N/A
1x Non-Aluminized	16.9	N/A
10x Aluminized	35.2**	35.2*
10x Non-aluminized	48.6*	37.4*
Caged		
1x Aluminized	22.2	N/A
1x Non-Aluminized	N/A	N/A
10x Aluminized	N/A	39.6*
10x Non-Aluminized	6.8	40.2*

* Rejects the hypothesis of equal means at the 95% Confidence Level when compared to controls.

** Rejects the hypothesis of equal means at the 90% Confidence Level when compared to controls.

SUMMARY

1) Two methods of calculating results are available when performing bioassays on oyster larvae: The Calabrese et al., (1973) method and the live-count surviving method.

2) A margin of error is associated with the injection of the oyster larvae innoculum which is not accounted for when using the Calabrese et al.,(1973) calculation methodology. This error is, however, accounted for when using the live-count surviving calculation methodology presented in this paper.

3) The live-count surviving method, on the other hand, does not take into account the percentage of larvae not recovered. The reason being that these larvae may have decomposed upon death or may not have been fertilized when injected.

4) Until more information is known about the injection error (which can then be introduced in the method of calculation) consideration must be used when interpreting data using both calculation methods.

APPENDIX B

Determination of Innoculum

EXPERIMENT I- Range Find

Chesapeake Bay Water Study

Fertilized Egg Counts per 1 ml sample

289
321
331
320
319
1580

Average used as innoculum =
1580/5 = 316 fertilized eggs

Synthetic Seawater Study

Fertilized Egg Counts per 1 ml sample

261
272
248
256
253
1290

Average used as innoculum =
1290/5 = 258 fertilized eggs

EXPERIMENT II - Uncaged & Caged Study

Fertilized egg counts per 1 ml sample

335
322
345
336
314
1652

Average = 1652/5 = 330.4 fertilized eggs

Injection = 330.4 x .91 ml = 300 fertilized eggs

Innoculum = 300 fertilized eggs per beaker

SECTION II - Nereis succinea

METHODOLOGY

The polychaete annelid worm, Nereis succinea, was chosen as the test animal since it represents the largest and most abundant annelid species found in the area subject to exposure within the Chesapeake Bay. The specimens were collected from the oyster holding tanks in CEL and placed in aquaria of flowing seawater. Glass and Tygon tubing, cut to approximately 3 inches in length, were placed in the aquaria for the worms to inhabit. After a period of about 10 days the worms which did not remain in the tubes were discarded. During the acclimation period the worms were fed daily with a high concentration of oyster feces and pseudofeces. The test chambers consisted of nine 10 gallon glass aquaria, divided in half with 54 μ mesh Nytex screening. The aquaria were filled with water pumped from the mouth of the Patuxent River, the same source of water from which the worms were collected and acclimated. The water was not renewed during the experimental period, however air was bubbled in one half of the aquaria.

Forty worms in their glass or Tygon tubules were taken from the acclimation chamber and placed in each aquaria; twenty in the half where the chaff was placed (caged) and twenty in the half without chaff (uncaged). The water was free to flow from one side to the other but the chaff was restricted to the caged side. The surface areas of the 10 gallon aquaria was 1.4 square feet. Therefore, the amount of 10x chaff used in each aquaria was 74.2 dipoles (53 dipoles per square foot). Each test was triplicated, three aquaria contained aluminized chaff, three contained non-aluminized chaff, and three were control chambers.

Water quality measurements on temperature, pH, dissolved oxygen, salinity and NH_3-N, were made daily from the uncaged section of a control and treatment aquarium for a period of four days. Behavioral observations were made daily from both sections of all aquaria for the same period of time.

The worms in their tubules were not physically distributed during the four or eight day test periods. Observations during this time were visually made. At the end of this period however each tubule was closely examined with a probe to expel the worm or detritis from the glass or plastic tubing. Worms were determined alive if activity was observed in movement of the parapodia, the usual form of locomotion. Conversely, they were classified as dead if no movement was observed and they usually had a gray coloration. Some tubules were

empty with no sign of a dead or live worm but usually with a black anaerobic substance. This could have been the remains of fecal material from the worms or completely disintegrated worm tissue. The vacant tubules were not included in the analysis since the causes could not be determined.

RESULTS

Two tests were run; one four day experiment, and one eight day experiment. These results are given in Tables 1 and 3. During the exposure periods the worms were observed leaving the tubules in both the control and treatment tanks. They were not more active in the latter stages of the experiment but their behavior appeared unpredictable throughout. Probably their excursions outside the tubules were in search of food since they are scavengers and food was definitely limited in the static system. Cannibalism was not observed, but could have occurred and may have accounted for some of the vacant tubules.

FOUR DAY EXPOSURE

The aluminized and the non-aluminized chaff floated on the surface of the water for a period of time depending on the amount of filament clumping. All chaff sank to the bottom by the second day. The chaff on the bottom came in direct contact with the worm tubules and was used by some worms to plug the ends of the tubules while they remained alive inside. At the end of the four day test period no dead worms were found in the aquaria. Many vacant tubes were found but were not included in the statistical analysis for reasons stated earlier. The statistical test (ANOVA) is based entirely on the live remaining worms at the end of the experiment. The results are given in Table 5. No statistical difference could be measured between worms in tanks of aluminized chaff, non-aluminized chaff, and control tanks. Each experiment was triplicated and tested in caged and uncaged portions of the tanks.

EIGHT DAY EXPOSURE

After termination of the four day exposure test, the worms were placed in the acclimation chamber for five days where they were fed. They were then placed in the test aquaria as previously described. In this experiment only 20 worms per tank or 10 in the caged area and 10 in the uncaged area were used. The same amount of chaff was used as in the first experiment (74.2 dipoles per tank).

These were placed in Davidson's Fixative for any future histological examination. Water quality measurements were taken periodically during the experiment and are given in Table 4.

Some mortalities occurred during this eight day exposure as compared to none in the first four day experiment. Mortalities in the caged sections of the aquaria averaged about 17% for the three replicates with aluminized and non-aluminized chaff. In the control tank, comparable to the caged section of the test aquaria, mortalities averaged 17% also. In the uncaged portions of the chaff aquaria mortalities averaged 7%, compared to 33% in the control aquaria.

It therefore appears that the percent of mortalities was about the same for tanks with the chaff, either aluminized or non-aluminized and either caged or uncaged. For some unexplanable reason the control aquaria had about twice the percentage mortality than the treatment aquaria.

Since no mortalities were observed in the four day experiment while in the eight day experiment only about 17% death occurred, it was believed that no measurable effects from chaff were seen. The mortalities probably could be attributable to starvation in the second experiment. Most of the dead worms were found outside their tubules, apparently in search of food. Some live worms also were found outside the aquaria crawling on the bottom or sides of the tanks.

The same statistical test of significance was run on this experiment as with the four day experiment using the live remaining worms. No significant difference could be measured (95% level), between the control and treatment chambers (Table 5).

TABLE 1

Results of the 4 day exposure to 10x chaff on the Polychaete worm, *Nereis succinea.*

	ALUMINIZED CHAFF TANK			NON-ALUMINIZED CHAFF TANK			CONTROL TANK		
	1	2	3	1	2	3	1	2	3
CAGED									
Alive	13	10	15	12	13	17	13	14	15
Dead	0	0	0	0	0	0	0	0	0
Vacant	7	11	5	9	7	3	7	6	5
%Mortality	0	0	0	0	0	0	0	0	0
UNCAGED									
Alive	17	16	13	14	13	17	15	13	18
Dead	0	0	0	0	0	0	0	0	0
Vacant	3	4	7	5	7	3	6	7	2
%Mortality	0	0	0	0	0	0	0	0	0
TOTAL									
Alive	30	26	28	26	26	34	28	27	32
Dead	0	0	0	0	0	0	0	0	0
Vacant	10	15	12	14	14	6	13	13	8

TABLE 2

Ranges of water quality in the control and experimental tanks for the four day chaff experiment with <u>Nereis succinea</u> .

	Temp (°C)	Salinity °/₀₀	D.O. ppm	pH	NH_3-N ug
Control	25.0 - 22.0	14.3 - 15.0	8.1 - 4.7	7.0 - 7.8	*Tr - .48
Experimental	25.0 - 22.0	14.2 - 15.0	8.1 - 4.9	7.0 - 7.8	*Tr - .51

*Tr = Trace

TABLE 3

Results of the 8 day exposure to 10x chaff on the Polychaete worm, *Nereis succinea*.

	ALUMINIZED CHAFF TANK			NON-ALUMINIZED CHAFF TANK			CONTROL TANK		
	1	2	3	1	2	3	1	2	3
CAGED									
Alive	6	6	6	6	7	6	9	8	5
Dead	1	1	1	0	1	2	0	1	2
Vacant	3	3	3	4	2	2	1	1	3
% Mortality	17	17	17	0	14	33	0	13	40
UNCAGED									
Alive	6	5	7	6	6	5	8	8	4
Dead	0	1	0	0	0	1	2	0	3
Vacant	4	4	3	4	4	4	1	2	3
%Mortality	0	20	0	0	0	20	25	0	75
TOTAL									
Alive	12	11	13	12	13	11	17	16	9
Dead	1	2	1	0	1	3	2	1	5
Vacant	21	7	6	8	6	6	2	3	6

TABLE 4

Ranges of water quality in the control and experimental tanks for the 8 day chaff experiment with _Nereis succinea_

	Temp. °C	Salinity °/oo	D.O. (ppm)	pH
Control	25.5 - 19.0	16.2 - 15.5	6.7 - 6-0	7.9 - 8.1
Experimental	25.5 - 19.0	16.2 - 15.1	6.8 - 6.2	8.0 - 8.1

TABLE 5

Results of statistical analysis (ANOVA) for the 4 and 8 day exposure testing the effects of 10x chaff upon the annelid worm <u>Nereis succinea</u>. Live remaining worms were used for analysis.

FOUR DAY EXPOSURE (CAGED)

	CONTROL	ALUMINIZED	NON-ALUMINIZED	D.F	F.
Mean	14.0	12.67	14.0	2	
Variance	.667	4.22	4.667	6	.3721

FOUR DAY EXPOSURE (UNCAGED)

	CONTROL	ALUMINIZED	NON-ALUMINIZED	D.F.	F.
Mean	15.3	15.33	14.66	2	
Variance	4.22	2.82	2.89	6	.0889

EIGHT DAY EXPOSURE (CAGED)

	CONTROL	ALUMINIZED	NON-ALUMINIZED	D.F.	F.
Mean	7.33	6.0	6.33	2	
Variance	2.88	0	.22	6	.928

EIGHT DAY EXPOSURE

	CONTROL	ALUMINIZED	NON-ALUMINIZED	D.F.	F.
Mean	6.66	6.0	5.66	2	
Variance	3.55	.67	.22	6	.349

CONCLUSIONS

1. Laboratory tests have revealed varying degrees of vulnerability of the species tested to chaff material.

2. Aluminized and non-aluminized chaff exhibit a significant effect on 0-48 hour oyster larvae (Crassostrea virginica) at the 10x concentration.

3. This effect based on Calabrese's et al., (1973) Calculation Methodology is a result of direct exposure to the chaff.

4. Aluminized and non-aluminized chaff exhibit no significant effect on Nereis succinea at the 10x concentration for test periods of 4 and 8 days.

RECOMMENDATIONS

1. Long-term exposure studies should be undertaken to determine whether toxic materials from chaff are accumulated to any degree in the food chain.

2. Design more comprehensive realistic laboratory tests to include the effects of mobility of chaff in the environment.

3. Perform field studies to determine if chaff accumulates in the environment.

4. Perform complete chemical analysis of chaff material to characterize its salt water chemistry.

5. Based on this study and the similarity in responses to toxic substances among different bivalve larvae we suggest that the use of chaff during shellfish spawning months should be discontinued until the above recommendations have been carried out.

LITERATURE CITED

SECTION I

Calabrese, A., R.S. Collier, D.A. Ne · and J.R. MacInnes, 1973. The Toxicity of Heavy Metals to Em /os of the American Oyster Crassostrea virginica, Marine Biology 18, 162-166.

Loosanoff, V. L. and H.C. Davis, 1963. Rearing of bivalve mollusks. In: Advances in Marine Biology, Vol. 1, London.

Standard Methods for Examination of Water and Wastewater 1976. 14th Ed. APNA-AWWA-WPCF. 1193 pp.

REFERENCES

Mangum, Charlotte P. 1969. Low temperature blockage of the feeding response in boreal and temperate zone polychaetes. Chesapeake Science 10(1): 64-68

Southward, A.J., and Eve S. Southward, 1972. Observations on the role of dissolved organic compounds in the nutrition of benthic invertebrates. III. Uptake in relation to organic content of the habitat. Sarsia 50:29-46.

Tenore, K.R. 1970. The macrobenthos of the Pamlico River estuary, North Carolina Water Res. Inst. Univ. of North Carolina No. 40, 112 p.